SOME ASPECTS OF THE ANATOMY AND CHEMISTRY OF FISH MUSCLE

ABSTRACT

Thesis submitted for the Degree of

DOCTOR OF PHILOSOPHY

IN

ZOOLOGY

to

The Aligarh Muslim University,
Aligarh

By

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M. Sc., M. Phil. (Alig.)

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DEPARTMENT OF ZOOLOGY
ALIGARH MUSLIM UNIVERSITY
ALIGARH

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ABSTRACT

Studies were made on various aspects of the chemical biology of freshwater teleosts. Since certain morphological peculiarities produce chemical heterogeneity, influencing considerably the results of the biochemical analyses, some investigations on the morphology of body musculature were also carried out.

The body musculature of the murrel, Ophicephalus punctatus, in its general architecture, was found to be typically of 'piscine type'. The myotomes were divisible into epaxial and hypaxial portions, each of which was further differentiated into two muscle bundles. The myotomes and myocommata were found to extend inward as far as the sagittal axis of the body. Their planes of interception were at various angles which varied with the region, becoming sharper towards the tail end of the fillet. The logarithms of the thickness of myotomes and the lengths of epaxial and hypaxial portions were positively related to the logarithms of the total body length of the fish. The thickness of myotomes declined progressively towards the tail region, while the proportion of connective tissue (myocommata) exhibited a relative increase. The quantitative analysis of the myotomic growth indicated that the growth of these structures in this species was in direct proportion to that of the body. The growth was age-specific, being rapid during the early life and declining gradually with advance in age. The growth rate of the anterior myotomes was faster than that of the more posterior ones. The ratios of their differential growth rates varied within a very restricted range.

Investigations on the distribution of some chemical constituents in the body musculature of O. punctatus revealed that the musculature was chemically heterogeneous. The concentrations of

protein, total fat, ash, total carbohydrates, glycogen, cholesterol, RNA and DNA in the muscles of the tail section were higher than those of the trunk. Water content maintained a reciprocal distributional characteristic with the fat.

From a comparative study of the distribution of some biochemical constituents in the dark, white muscles and the liver of the cat-fish, Clarias batrachus it was found that the concentrations of protein, water and ash were highest in the white muscle and lowest in the liver. In the dark muscle these constituents occurred in intermediate quantities. Liver appeared to be the richest source of fat, glycogen, cholesterol, RNA and DNA, followed by the dark muscle, while the white muscle remained poorest in these substances. The concentration of water in the three tissues maintained an inverse progression with that of the fat.

Examination of the changes occurring in the proximate biochemical composition of the body muscles of L. striatus, during growth, revealed that protein and fat contents declined in specimens of successive year-classes, from 0+ - 3+ due to their mobilisation towards growing body tissues and the gonads, but registered an increase in fishes of 4+ year-class, perhaps as a result of recovery from active growth, coupled with the consumption of more and highly nutritive food. No marked changes occurred in the succeeding growth period. Water content varied inversely with that of fat. Ash content did not seem to undergo any regular change.

In L. punctatus the pattern of changes in the concentrations of RNA and protein was strikingly similar. After maintaining a decline in fishes of 1+ age-group, the two constituents increased thereafter. Irrespective of the systematic relationships, the growth-cum-biochemical cycles in fish seemed to be influenced by a multitude of factors of intrinsic and extrinsic nature. The RNA and protein concentrations in the body musculature of this species were found to be closely related to its 'biological condition'.

Deprivation of Heteropneustes fossilis from food seemed to result in a progressive decline in body weight and the 'fillet condition factor'. This was invariably an outcome of the utilisation of the various architectural constituents of the body, principally the protein and fat. Mobilisation of these high energy substances led to calorific loss from the body. The percentage of water maintained a reciprocal relationship with that of fat. Ash also registered a decline during starvation. In the absence of any replenishment from exogenous sources due to food deprivation, the breakdown of endogenous protein of the tissue resulted in a negative nitrogen equilibrium.

Growth in the pre-maturity phase of the ovary, namely, Labeo calbasu and Puntius sarana was characterised by an increase in RNA and an apparent decrease in DNA per unit weight of both the dark and white muscles. This decline in DNA concentration was compensated, to some extent, by the synthesis of more DNA in the cells. The possible factors governing these changes in the nucleic acids have been discussed. The dark and white muscles seemed to differ in their growth rates and DNA synthesis.

In C. batrachus and H. fossilis, the higher activity of acid and alkaline phosphatases in the dark muscle than the white, implied greater metabolism of phosphomonoesters, carbohydrates and phospholipids. On the contrary, more activity of 5'-nucleotidase in the white muscle in comparison to the dark one, was perhaps to regulate the cycle of inorganic phosphorus and adenosine monophosphate which is more active in the mechanical tissue. The inhibition of alkaline phosphatase activity of dark, white muscles and the liver of these species by certain amino acids (L-phenylalanine, L-methionine, L-arginine and L-valine) seemed to be species-specific. The tendency of differential inhibition of the enzyme activity was, however, found to be tissue-specific.

Studies on the carbohydrate metabolism in C. batrachus revealed that muscular activity induced glycolysis in the dark and white

muscles. This was evidenced by a decline in the glycogen content, with a consequent increase in pyruvic acid level, followed by marked rise in the lactic acid concentration. Although the glycolytic activity in both the types of muscles was fundamentally in accordance with the Embden-Meyerhof-Parnas (EMP) scheme, comparatively little difference in the glycogen level of the dark muscle in the pre- and post- exercise states indicated towards a faster resynthesis of this polysaccharide in the dark muscle. The blood levels of these metabolites varied with the sequence of their turnover in the muscles. Glycogen degradation did not seem to occur in the liver. However, the transport of these substances via blood was responsible for the dynamics of their change in the liver. The level of 'excess lactate' in the blood increased steadily with the duration of muscular activity, due to an increase in the ratio [illegible]. The details of the carbohydrate metabolism in _____ in relation to muscular activity were basically similar to those in _____ except that the glycolytic activity also occurred in the liver.

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C O N T E N T S

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GENERAL INTRODUCTION

The study presented here is the biology of some freshwater fishes with relevance to chemical composition. This aspect was selected in view of the profound influence which the various life processes exert on the chemistry of fish. Such investigations invariably become of utmost significance from the standpoint of food science and it is this nutritional approach of study which gives special status to fish chemistry, in contrast to biochemical observations on some other animals, having no bearing on human nutrition.

Although the significance of relating the biological phenomenon of fish to its chemical composition has been recognized by fishery biologists, the subject of 'Chemical Biology' of fishes has not received the importance it deserves for. Efforts have, therefore, been made in the present study to bring unity between these two disciplines of Ichthyology. Before doing so, an attempt has been made to assess the degree of impact the anatomical heterogeneity of fish musculature is brought to bear on the concentrations of various chemical constituents of the muscles. Furthermore, these investigations provided an opportunity of having a better glimpse of the anatomy of fish musculature in relation to the distribution of its chemical constituents and the results warranted against taking the unspecified parts of fillets

for chemical analysis, since a mere faulty sampling technique could have the potential of easily jeoparadising any information which might have emerged from the chemical analyses of fish tissues.

It seems rather unfortunate that despite the very anatomical location and constancy, no quantitative studies on the axial musculature of fish have been made as yet. An extensive study had, therefore, been undertaken on the anatomical aspects of the quantitative myology of a freshwater teleost, Ophicephalus punctatus Bloch. In a metamerically defined animal like fish, it was thought necessary to analyse the pattern of arrangement of myotomes and myosepta, which if traced in detail, is reported to be rather complex (Ganguly and Nag, 1964). A study of the general organization of the myotomes formed the basis of deriving some generalisations as to the classification of the myotomes into architectural type, their regional differentiation and relationship with the axial skeleton of the fish. In addition to evaluating the logarithmic relationships between the thickness of myotomes and body length of the fish through regression equations and 'myosomatic indices', attempt was made to establish the relationships between the lengths of each of the epaxial and hypaxial portions of the myotomes with the body length of the fish.

Insofar as the growth of the muscle fibers is accompanied by changes in the nucleic acid content, mainly the DNA, per unit

weight of the tissue (Love, 1970), the differential in the growth rates of the myotomes of different body locations can bring about marked divergence in the values of RNA in the given weight of the muscle sample, from varied anatomical regions of the musculature. This necessitated the analysis of the pattern of growth of the myotomes of different regions. In the present study, the fourth and twenty-first myotomes were selected for this purpose. The criteria being to study the growth characteristics of the anterior and posterior myotomes with its bearing on the RNA concentration. It should be emphasized, rather strongly, that the selection of these particular myotomes had no other role to serve.

The turnover of various biochemical constituents during the growth period of fish was also investigated. The deposition of some chemical constituents like protein, fat, etc., left over as extra calories, forms the very basis of growth. In the manipulation of growth the variations originate from the interaction of intrinsic (physiological) and extrinsic (ecological) factors, inasmuch as it is the unity between the species and its environment that sustains the process of growth. Although the application of the classical concept of growth, using change in wet weight furnishes a relatively simple method of assessment, it has its limitations because it precludes any changes in the proximate body composition (Hilmi and Beamish, 1974). Many workers have used the levels of protein, fat, as also the energy

content, to express fish growth, but the criterion of protein deposition has been preferred over that of carbohydrate and fat because these two chemical constituents are considered to be only temporary stores of energy, undergoing rapid changes, as compared to protein levels which are relatively stable. Following the establishment of the relationship between the nitrogen content and protein value and the elucidation of a protein factor (6.25), the increase in the level of total nitrogen was regarded to be synonymous with a direct increase in the protein content. In the present investigations the data pertaining to the principal body constituents (protein, fat, water and ash) were examined to further document the changes in the body composition with growth, in Labiosphalus striatus Bloch. To find the inter-specific differences, if any, in the growth-cum-biochemical cycles, another closely related species, Labiosphalus punctatus was also examined.

After the elucidation of the role of nucleic acids in protein synthesis it was considered desirable to find if the quantitative estimates of nucleic acids could be used for the assessment of growth in fishes. A glance at the recent literature indicates that attempts have been made towards the prediction of growth rate and measurement of biomass production of marine phytoplankton and zooplankton through the nucleic acid determinations (Autoliffe, 1965; Holm-Hansen et al., 1968). Bulow (1970) has indicated the usefulness of nucleic acid determinations in the detection of 'recent' growth rates of fish.

The biochemical studies on the age and growth of fish from biological standpoint, were further extended to the length-weight relationship and condition factor. The biochemical and physiological changes occurring during starvation of fish were also investigated, as the deprivation of food can influence the growth and 'condition' of the fish. Since the present observations were based on the edible portion, the fillet, which constitutes the main bulk of the body of a fish, the changes in the fillet weight were, therefore regarded as the most relevant and informative parameter. Thus, consideration was given to the 'fillet condition factor', a term proposed by Wilkins (1967), in the context of the study on changes in body weight, principal chemical constituents, energy content and nitrogen balance, during starvation of the cat-fish, Heteropneustes fossilis (Bloch

The remaining part of the thesis reports the biochemical and physiological observations on the dark and white muscles of fish. A survey of literature reveals that this subject has aroused quite a lot of curiosity and continues to attract the attention of many recent investigators. Before any experimental work was carried out some early workers believed that the darkening of the muscle was merely due to blood (Needham, 1926). This assumption was rejected on the ground that even when the blood vascular system was washed out the dark muscle still remained dark. Subsequent authors directed their attention towards the biochemical and physiological studies. Some of the pioneer workers in this field are Ansaura and Hashimoto (1954),

Nanakawa et al. (1957), Saito et al. (1959), Buttkus (1963), Iyer et al. (1963), Gupta and Lori (1963), Sallgren and Mathieson (1966), Ackman and Cormier (1967), Lin et al. (1974), George (1975). Large number of chemical constituents were analysed and it was found that the differences were very formidable and would cause error if the mixed tissue was used for sampling (Love, 1970). What further complicated the issue was that some constituents like cholesterol, glycogen, histidine, niacine, protein and water, have been listed as being both more concentrated and less concentrated according to different workers (Love, 1970). The subject became still more controversial after the publications of Brockkan (1956, 1959) and of Lori et al. (1956) on the analyses of vitamins of the B-group. These authors noticed that the concentrations of these vitamins in the dark muscle were considerably different from their concentrations in the white muscle but at the same time resembled their concentrations in the liver of the same fish. From this they deduced that the function of the dark muscle could be similar to that of the liver. Support to this 'liver concept' was extended by the neurophysiological observations of Barets (1961) and electrophysiological studies by Nishihara (1967). Further investigations by Wittenberger and Gross (1961), reporting a negative correlation between the degree of development of dark muscle and the size of the liver, favoured the 'liver concept'. Investigations contradicting the 'liver concept' and revealing similarities between the dark muscle and the heart have also been reviewed by Love (1970).

In the light of conflicting reports mentioned above, a balanced assessment of the entire situation seemed rather difficult, since there were contradictions in findings of like experiments carried out by the protagonists of the rival theories (Love, 1970).

In an attempt to find the affinities of the dark muscle with the white, on the one hand and with the liver, on the other, investigations were carried out on the concentrations of some chemical constituents in the three tissues of Clarias batrachus (Linn) a common freshwater cat-fish.

In addition to the distribution of RNA and DNA in the dark and white muscles, the pattern of their turnover in the two types of muscles during the process of growth in the pre-maturity phase was also elucidated. Some new informations which emerged from these studies have been discussed at length.

For enzymatic studies the acid and alkaline phosphatases, and 5'-nucleotidase were selected. The investigations on the distribution of these enzymes were carried out only on the dark and white muscles.

To throw more light on the functional role of the dark muscle in relation to mechanical activity, two species of fish, namely, Clarias batrachus and Ophiocephalus punctatus, the former having conspicuous dark muscle and the latter having highly

reduced dark muscle, were selected and the metabolic activity in their muscles and the liver investigated. The results were compared and the effect of the reduction of dark muscle in Chiloscyllium punctatum was taken to indicate the role dark muscle could play in the mechanical activity of the fish. Metabolism of carbohydrates formed the basis of these investigations.

PROCEDURE AND METHODOLOGY

METHODS OF CATCHING.

Fishes forming the basis of present investigations were captured by cast-nets from the freshwaters of Aligarh (Lat. 27° 54' 30" N, Long. 78° 4' 25" E) and transported to the laboratory. Unless stated otherwise the specimens were released into aquaria (95 x 35 x 45 cm) provided with water at a particular temperature. The water supply was so adjusted as to maintain a definite concentration of dissolved oxygen.

At the time of investigations, specimens were taken out, killed by decapitation and examined in various ways for morphological studies, while for the biochemical analyses, the desired tissues were immediately removed, freed from skeletal elements and in some cases macerated in an electric grinder before being processed for chemical estimations.

FOR MORPHOLOGICAL STUDIES ON THE BODY MUSCLES OF OPHIOCEPHALUS PUNCTATUS.

(1) Removal of the skin and measurements of the thickness of myotomes

Ophiocephalus punctatus were removed from the aquaria and measured to the nearest millimeter. After decapitation the specimens were skinned from the point of severance of the head to the tail.

Care was taken to avoid any injury to the muscles. The fourth and twenty-first myotomes (as counted from the anterior region) were selected for measurements of their thickness (distance between two adjacent myocommata). The measurements were carried out on the skin side of the fillet, above the lateral septum.

(2) Filleting:

The fillets were removed by a sharp knife inserted in the region of caudal peduncle, deep up to the vertebral column and then pulled carefully towards the anterior side as far as the commencement of the head region.

(3) Sectioning:

The region of maximum girth, adjacent to the dorsal fin, was selected and sectioned exactly at right angles.

(4) Measurements of the epaxial and hypaxial portions, median skeletogenous and lateral septa:

In the cross-sections, thus cut, various measurements of the components of the myotomes were made. The epaxial portion was measured all along the edge of the cut section, from the region of the dorsal septum to the lateral septum. The hypaxial portion was measured in the similar way, all along the edge of the cut section, from the lateral septum to the linea alba.

The distance the lateral septum traverses in passing inward was measured from the surface to the median skeletogenous septum.

The dorsal portion of the median skeletogenous septum was measured from the lower extremity of the vertebral centrum straight up at right angles up to the region of the commencement of the muscles of the dorsal fin ray.

FOR STUDIES ON THE CHEMICAL HETEROGENEITY OF BODY MUSCULATURE OF FISH.

Ophiocephalus punctatus and Clarias batrachus of the body length 18-20 cm and 20-24.5 cm respectively were collected and released into aquaria which were maintained at a temperature of 25 ± 2°C and a dissolved oxygen concentration of 6 ± 2 ppm. The fishes were allowed to rest for 24 hours before sampling.

Muscle samples of O. punctatus were taken from the epaxial portions of the trunk and tail regions of the fillet.

In C. batrachus the sample of white muscle was excised from epaxial portion of trunk, adjacent to dorsal fin, while dark muscle was obtained from the region of lateral line in the same region of trunk. Liver was removed and cleared of the adhering fluid.

FOR STUDIES ON THE BIOCHEMICAL COMPOSITION OF FISH MUSCLE IN RELATION TO GROWTH, 'BIOLOGICAL CONDITION' AND STARVATION OF FISH

(1) In relation to growth:

For biochemical studies on growth, two species of murrels, namely, Ophiocephalus striatus and Ophiocephalus punctatus of various sizes were selected and freed into aquaria running at a temperature of 15-16°C and a dissolved oxygen level of 5-6.5 ppm. Age determination was carried out by counting the number of annuli on their scales obtained from the trunk region between the place of the origin of dorsal fin, above the lateral septum.

Sample of muscle (white) was procured from the epaxial portion of the trunk region adjacent to the dorsal fin.

(2) In relation to 'biological condition':

Live specimens of Ophiocephalus punctatus which formed the basis of this study belonged to 20-30 cm body length. The specimens were introduced in aquaria (temperature, 11 ± 1°C; dissolved oxygen, 6.5-7.5 ppm).

After measuring its length in the intact fish, the fillet from each specimen was removed from the point of severance of the head to the caudal peduncle and freed from the skin and bones. It was immediately weighed on an electrical balance sensitive up to 0.001 g. The 'fillet condition factor' (C) was

calculated by the following formula as proposed by Wilkins (1967):

$$C = \frac{W}{L^3} \times 1000$$

where, W was the weight of the fillet (g), and (L) was the total length (cm)

For chemical estimations, muscle sample was taken from epaxial portion of trunk.

(3) In relation to starvation:

For proximate body composition, nitrogen balance and energy losses in Heteropneustes fossilis.

For these investigations Heteropneustes fossilis of the sizes 17.0 to 24.7 cm were maintained in the aquaria. The water temperature and dissolved oxygen concentration varied from 13.5 - 16.5°C and 6-8.5 ppm, respectively.

Modified collar tags used in the present study consisted of a rectangular plate of Ivory paper (9 x 6 cm) covered over by water-proof, transparent self-adhesive tape. The plate was pierced by a filamentous wire which formed a loop around the trunk region adjacent to the dorsal fin. The use of tags was meant to record the decline in the weight during successive periods of starvation.

After making analyses on fresh, unstarved fishes, subsequent analyses were made on specimens taken out after 10, 20, 30, 40 and 50 days of starvation.

At the time of investigation, fishes were removed from aquaria, weighed, measured and killed by sharp blow on the head. Methods for the removal of fillets, measurement of their length and weight, together with the calculation of fillet condition factor were the same as described earlier. Each fillet was chopped, mixed in an electrically driven macerator and processed for various assays.

Although attempt was made to use fishes of a similar size and weight, the variations did occur and warranted a mathematical adjustment. Likewise, the loss in the weight and principal chemical constituents was made to correspond to that of a fish of 50 g initial weight.

FOR BIOCHEMICAL AND PHYSIOLOGICAL STUDIES ON THE DARK AND WHITE MUSCLES OF FISHES.

(1) <u>For studies on nucleic acid turnover during growth in pre-maturity phase of carp:</u>

Freshly killed specimens of two species of teleosts were brought to laboratory and their body lengths measured to the nearest mm. Specimens were classified into two size groups: Group I consisted of juveniles, Group II comprised, fishes that had grown to twice the body length of juveniles but which were

not yet mature. The pre-maturity phase was identified on the basis of the usual I.C.E.S. (International Council for the Exploration of the Seas) scale.

Method for the sampling of dark and white muscles was the same as already described.

(2) For studies on enzymes:

For these investigations, two species of cat-fishes, Clarias batrachus and Heteropneustes fossilis of body length 17-21 cm were selected and released into aquaria, supplied with water at 15 ± 1°C and the dissolved oxygen concentration was maintained at 9 ± 1 ppm.

White muscle was taken out from the epaxial portion adjacent to dorsal fin, while dark muscle was obtained from the lateral line.

For studies on amino acid inhibition of alkaline phosphatase activity, liver was also analysed, in addition to dark and white muscles.

(3) For studies on the carbohydrate metabolism:

Two species of teleosts, namely, Clarias batrachus (body length : 24-28 cm), Ophiocephalus punctatus (body length : 20-23 cm) were used for the present investigations.

Fishes were allowed to rest in the aquaria for 48 hours before sampling. The flow of water was so adjusted as to

maintain a dissolved oxygen concentration of 8-12 ppm. The temperature of water remained at 29 ± 2°C.

After carrying out investigations on unexercised fishes, the other specimens were towed and forced to swim for various durations (5, 10, 15, 20 minutes) at a known speed (90 cm/sec). Throughout the course of these investigations, the fishes were exercised at a fixed time (early in the morning) as suggested by Beamish (1968) to minimise the possible effect of diurnal variations on the concentrations of the various metabolites.

After exercise, the fishes were killed by decapitation and the desired samples were immediately removed. Techniques for the sampling of dark, white muscles and liver were the same as mentioned earlier. In Ophiocephalus punctatus, dark muscle could not be examined as its proportion was highly reduced, forming a very narrow lateral strip. Blood was obtained from the dorsal aorta in the region of caudal peduncle.

METHODS OF ESTIMATION

WATER

For the determination of water content in tissues a known amount of fresh sample (2-5 g) was taken in a pre-weighed silica crucible and placed in an electric oven running at 100°C. for about 18-20 hours (A.O.A.C., 1960) till the tissue became completely free from water. The crucible was then removed from the oven and allowed to cool in a desiccator and reweighed. The

entire process was repeated several times until a constant weight was obtained. The loss of weight gave an index of water from which the percentage of water was calculated on wet weight basis.

ASH

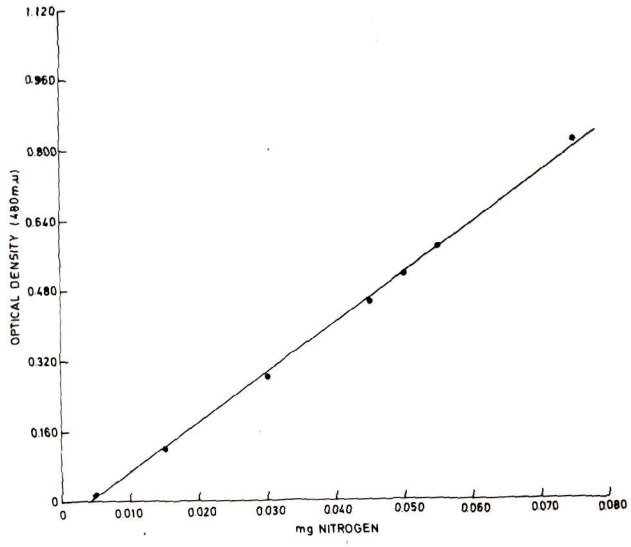
A known weight of fresh sample (2-5 g) was placed in a silica crucible, dried in an electric oven at 100°C, and ignited at a temperature of about 500-600°C, till the sample became completely white and free from carbon. The operation was continued till a constant weight was obtained. The amount of ash was expressed as percentage on wet weight basis.

TOTAL FAT

Total fat in tissues was extracted by the Soxhlet extraction method, using petroleum ether (B.P. 40-60°C) as the solvent. The use of this solvent in the routine analysis was found to be relatively quicker and effective.

Fresh tissues were dried at 100°C in an electric oven, mixed and ground thoroughly. A weighed quantity of the dried sample was taken in whatman fat extraction thimble and plugged with cotton wool. The extraction was carried out with petroleum ether, for about 10-12 hours in Soxhlet apparatus. Distillation of the solvent from the fat extracts was done by keeping the receiving flask on the water bath. The flask was then placed in an oven for complete removal of the solvent trace. Finally, the flask was then cooled in a desiccator and weighed. For

Fig. 1. Standard curve of NITROGEN.


1.120
0.960
0.800
0.640
0.480
0.320
0.160
0
OPTICAL DENSITY (480mμ)
0
0.010
0.020
0.030
0.040
0.050
0.060
0.070
0.080
mg NITROGEN

ensuring a complete extraction of fat from the tissue samples, the process was repeated until no further increase in the weight of the receiving flask occurred. The increase in the weight of the flask gave the quantity of fat extracted from the known weight of tissue sample. The amount of fat was calculated on wet weight basis and expressed as percentage.

PROTEIN

The determination of protein in various tissues was made by a slight modification of Wong's (1925) micro-kjeldahl method. A known quantity of fresh tissue (0.1 g) was digested in 5.0 ml of nitrogen-free 1:1 sulfuric acid. Potassium persulphate (saturated solution) was used as an oxidising agent. The digestion which converts all the nitrogenous materials present in the tissue into ammonium sulfate was carried out till the solution in the flask became water clear. The digested solution was diluted to 50 ml with distilled water. A known aliquote of this solution was then directly nesslerized by adding Hock and Benedict Nessler's reagent (see Hawk et al., 1954). After about 10 minutes of standing at room temperature the color intensity was read on a Bausch and Lomb Spectronic 20 spectrophotometer at 480 mμ wave-length, after adjusting the instrument to zero density with the blank. The intensity of the color developed was proportional to the amount of ammonium sulphate contained in the solution. The readings obtained for the various samples were read off against a standard calibration curve (Fig. 1) which was prepared by taking readings of a series of different

dilutions containing known amount of nitrogen. This method gave a direct reading of the amount of total nitrogen present in the sample. This quantity of total nitrogen was multiplied by the protein factor (6.25) to get the protein value. The values were recorded as percentages on wet weight basis.

In certain experiments, protein was also estimated following the procedure of Lowry et al. (1951). A highly purified, processed bovine serum albumin served as the standard protein for making comparisons. Tissue sample (100 mg) was homogenized in 1.0 ml of distilled water and centrifuged. The protein was precipitated by adding two volumes of 10% trichloroacetic acid (TCA). The supernatant was discarded and the pellet was washed twice with 95% ethanol. The sediment was treated with 1.0 ml of 1N sodium hydroxide and the content heated for 15-20 minutes in a boiling water-bath, to digest the tissue residue. After its digestion the tubes were cooled to room temperature and the volume of the content raised to 10 ml with distilled water and mixed. An aliquot of 0.1 ml was taken from this mixture and made to a volume of 2.0 ml with distilled water. One milliliter of carbonate-copper solution was then added, mixed and the contents allowed to stand for 10 minutes at room temperature. This was followed by the addition of 0.1 ml of Folin reagent and mixing. A blank was also prepared by taking 0.1 ml of a mixture containing 1 part of 1N sodium hydroxide and 9 parts of distilled water. This aliquot of 0.1 ml was then treated in the manner described for the experimental.

Fig. 2. Standard curve of PROTEIN.

After an incubation of 30 minutes at room temperature the color was read on Spectronic 20 spectrophotometer at 500 mμ wave length. A standard curve was prepared relating the optical density to micrograms of protein (Fig. 2) with processed bovine serum albumin as the standard.

TOTAL CARBOHYDRATES

The percentage of total carbohydrate was calculated by subtracting the sum of the percentage values of water, fat, protein and ash from 100, as suggested by Jafri et al. (1964). Values were recorded on wet weight basis.

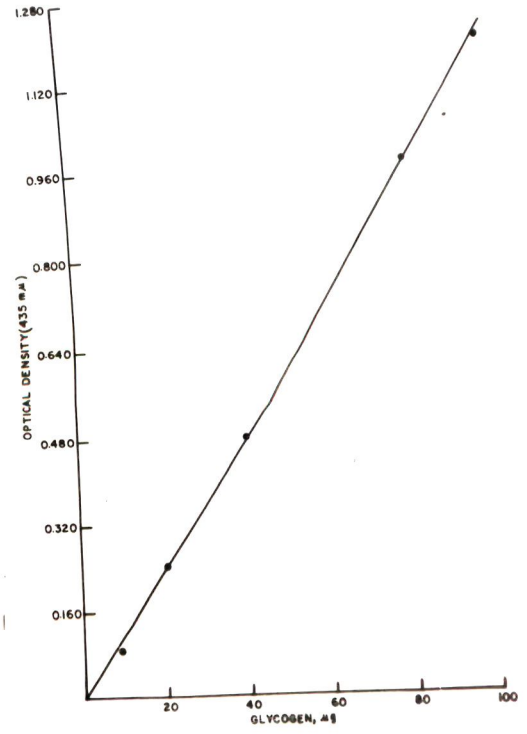
ENERGY VALUES

The energy value in terms of calories was calculated by using the factors 4.1 for protein and the same for carbohydrate, while 9.3 for fat, as proposed by Jafri et al. (1964).

GLYCOGEN

Glycogen was extracted from the tissues according to the procedure followed by Ashman and Reed (1973). Sample of 0.5 g of tissue was quickly removed from the fish and homogenised in 5.0 ml of 1.5% potassium hydroxide. The homogenate was centrifuged at 3,000 r.p.m. for 15 minutes. The supernatant was discarded and the pellet was suspended in 5.0 ml of 1.5% potassium hydroxide, and placed in a boiling water-bath for 30 minutes. Thereafter, the samples were cooled to room temperature and 5.0 ml of absolute methanol was added to precipitate the glycogen. The contents were mixed and centrifuged again

Fig. 3. Standard curve of GLYCOGEN.

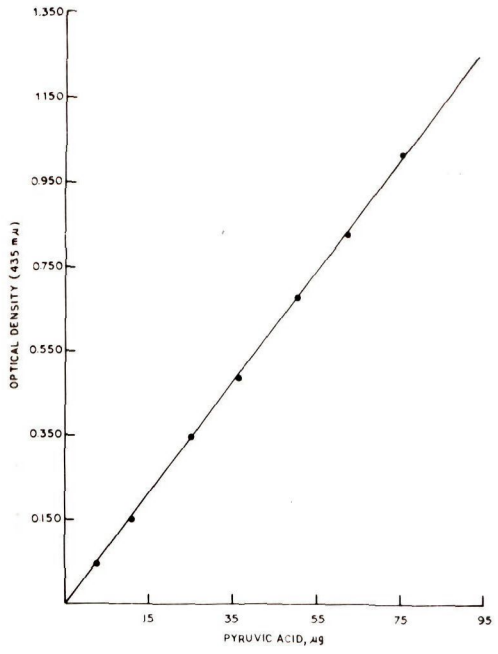

1.280
1.120
0.960
0.800
0.640
0.480
0.320
0.160
OPTICAL DENSITY (435 mμ)
20
40
60
80
100
GLYCOGEN, μg

for 15 minutes at 3,000 r.p.m. The supernatant was discarded and residue was resuspended in 5.0 ml of 1N-hydrochloric acid. The contents were heated for 30 minutes in a boiling water-bath. The solution was then filtered and the glycogen was determined in the filtrate by the method of Montgomery (1957). A known volume of aliquot was raised to 2.0 ml with distilled water, and 5.0 ml of concentrated sulfuric acid was added with constant shakings. The tube then received 0.1 ml of 80% (w/V) phenol and the contents were mixed thoroughly. A blank was also run by taking distilled water instead of glycogen extract and treate in a similar way. After allowing the tubes to stand at room temperature for 30 minutes, the color intensity was read at 490 mμ wave length, on a Bausch and Lomb Spectronic 20 spectrophotometer, after it was adjusted to zero density with the blank The values were compared with a calibration curve prepared by relating the optical density to different concentrations of standard processed glycogen (Fig. 3). The concentrations of glycogen were expressed as mg/100 g wet weight basis.

PYRUVIC ACID

Of the various methods used for the determination of pyruvic acid, the most sensitive is the one which is based upon the reaction of this acid with nitrophenyl-hydrazine (Friedemann and Haugen, 1943). In this method the hydrazones formed by the reaction with 2,4-dinitrophenyl hydrazine are extracted by benzene, re-extracted by 10% sodium carbonate and determined photometrically after the development of color by 1.5N sodium hydroxide.

Fig. 4. Standard curve of PYRUVIC ACID.


1.350
1.150
0.950
0.750
0.550
0.350
0.150
OPTICAL DENSITY (435 mμ)
15
35
55
75
95
PYRUVIC ACID, μg

A 3.0 ml of sample of 10% protein-free filtrate was transferred to a clean, dry test tube. A reagent blank containing 3.0 ml of TCA was also run. All the tubes were incubated for 10 minutes in a water-bath maintained at 25°C. After incubation, 1.0 ml of 2,4-dinitrophenyl hydrazine reagent was added to each tube. The incubation was continued at 25°C for exactly 5.0 minutes, after which 3.0 ml of benzene was added. The contents were mixed thoroughly and centrifuged at 3,000 rpm to facilitate the separation of phases. By the help of separating funnel the two layers were separated and the lower aqueous layer was discarded. The upper benzene layer was then transferred to a dry test-tube and 6.0 ml of 10% sodium carbonate solution was added from a dry pipet and the contents were again mixed thoroughly and centrifuged to complete the separation of the two layers. The layers were again separated through the separating funnel: the upper solvent (benzene) layer was discarded and 5.0 ml of the lower carbonate layer was taken in a test-tube held in a water bath at 25°C. Finally, 5.0 ml of 1.5N sodium hydroxide solution was added to each tube and mixed. After an incubation of 5-10 minutes the color intensity was read on Spectronic 20 spectrophotometer at 435 mμ wave-length, after setting the instrument to 100 % transmission with the blank. The values were read off against a standard curve prepared by relating the optical density to various concentrations of standard pyruvic acid (Fig. 4). Pyruvic acid concentration was calculated on wet weight basis, as mg/100 g. for tissue and as mg/100 ml for whole blood.

LACTIC ACID

Lactic acid in various tissues and blood was determined according to the method of Barker and Summerson (1941). The procedure consisted of the following steps:

Step 1. Removal of protein:

A 10% protein-free filtrate of the sample was prepared by treatment with 10 % TCA.

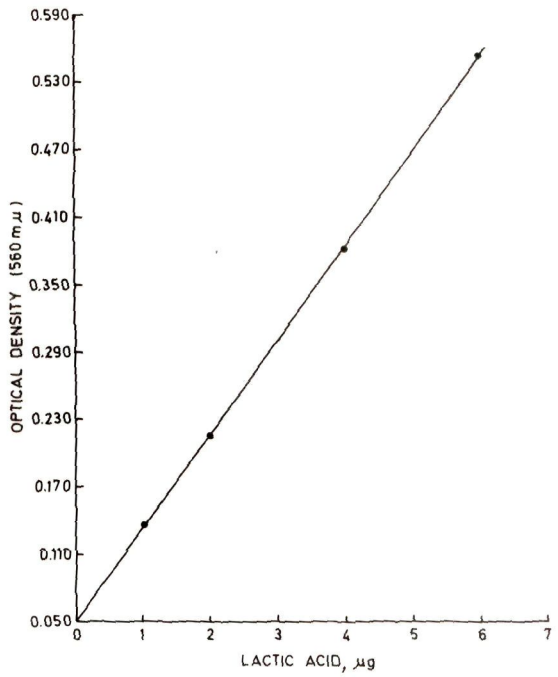
Step 2. Treatment with copper and calcium:

A measured volume (0.25 ml) of the protein-free filtrate was treated with 1.0 ml of 20% copper sulfate solution and the volume was made to 7 ml with distilled water. Approximately 1 g of powdered calcium hydroxide was added, the test-tube capped and shaken vigorously. The mixture was allowed to stand at room temperature for at least 30 minutes, with occasional shaking, and then centrifuged for 10 minutes at 2,000 rpm. The treatment with copper and calcium resulted in the removal of not only the glucose and other interfering substances but also of any protein remaining in the solution.

Step 3. Formation of acetaldehyde:

An aliquot (1.0 ml) of the supernatant obtained from step 2 above, was withdrawn carefully, transferred to a clean test tube and treated with 0.05 ml of 4% copper sulphate solution. The tube was then chilled in ice for about 10 minutes and thereafter 6.0 ml of concentrated sulphuric acid was added slowly

Fig. 5. Standard curve of LACTIC ACID.


0.590
0.530
0.470
0.410
0.350
0.290
0.230
0.170
0.110
0.050
OPTICAL DENSITY (560 mμ)
0
1
2
3
4
5
6
7
LACTIC ACID, μg

from a pipet with constant mixing. The tube was then placed in a boiling water-bath for 5 minutes, removed and cooled, thoroughly to below 20°C, first in the running water and then in ice.

Step 4. Development of color:

After cooling the contents, 0.1 ml of p-hydroxy diphenyl reagent was added and the precipitated reagent dispersed throughout the acid, as quickly and uniformly as possible, by vigorous shaking. The tube was placed in a beaker containing water at 30°C and allowed to stand for at least 30 minutes. The precipitated reagent was redispersed by shaking at least once during this incubation period. The tubes were then heated for 90 seconds on a boiling water-bath and finally cooled in cold water.

Step 5. Measurement of color:

The color intensity was read on a Bausch and Lomb Spectronic 20 Spectrophotometer at 560 mμ. A calibration curve was prepared by relating the optical density to various concentrations of standard lithium lactate (Fig. 5). The results were reported as mg/100 g tissue on wet weight basis and as mg/100 ml for whole blood.

The level of excess lactate was calculated by the following equation as proposed by Huckabee (1958):

$$XL = (Ln - Lo) - (Pn - Po)(Lo/Po)$$

where, XL = excess lactate, ml/liter of blood, Lo = concentration of lactate at rest, in ml/liter blood, Ln = concentration of

lactate at time n, in mM/liter blood, Po = concentration of pyruvate at rest, in mM/liter blood, Pn = concentration of pyruvate at time n, in mM/liter blood.

CHOLESTEROL

The amount of total tissue cholesterol was determined by the method of Heinhold and Thiele, as given by Hawk et al. (1954).

A known weight (0.1 g) of the tissue was placed in a small mortar containing about 8 g of anhydrous sodium sulphate and mixed uniformly. The sample was then dried in an electric oven at 100°C for 10-14 hours, cooled in a desiccator, pulverised transferred into a whatman thimble and cotton plugged. The thimble was then transferred into a soxhlet apparatus. The extraction of cholesterol was then carried out for 3 hours, using moisture-free, redistilled chloroform as the solvent. The extract was allowed to cool and transferred with rinsings to a 25 ml volumetric flask. The volume was then raised to the mark with chloroform and mixed well. From this, an aliquot of 10 ml was taken and 2.0 ml of the freshly prepared acetic anhydride - sulphuric acid reagent was added.. A 10 ml of the standard solution of cholesterol in chloroform, containing 0.8 mg of cholesterol, was treated in the same way. A blank was also prepared by taking 10 ml of chloroform alone and treating in the same way with the acetic anhydride sulphuric acid reagent. After thorough mixing, the tubes were placed in

a dark container maintained at 25°C for exactly 30 minutes for color development. The solutions were then transferred to dry cuvettes and the color intensity of the experimental and standard solutions was measured at a wave length of 660 mμ, on a Bausch and Lomb Spectronic 20 Spectrophotometer, after setting the instrument to zero density with the blank.

Cholesterol in the sample was calculated, using the equation:

$$\frac{\text{Optical density of unknown}}{\text{Optical density of standard}} \times 0.8 \times \frac{100}{0.4} = \text{mg cholesterol per } 100 \text{ g of tissue.}$$

From this value the amount of total cholesterol per 100 g of fresh tissue was calculated.

ACID AND ALKALINE PHOSPHATASE ACTIVITY

For the determination of phosphatase activity, a 2% homogenate was prepared and centrifuged at 3,000 rpm and 1.0 ml of the supernatant was taken for enzyme assay.

For the estimation of alkaline phosphatase activity the buffered β-glycerophosphate substrate (pH 9.4) of Bodansky, as suggested by Hawk et al. (1954), was used and the reaction mixture was incubated for 1 hour at 37°C, after which the reaction was stopped by the addition of 30% TCA. The liberated inorganic phosphate (pi) in the deproteinised sample was determined by the method of Fiske and Subbarow (1925). The color

intensity was measured on a Bausch and Lomb Spectronic 20 spectrophotometer at 680 mμ wave length. The enzyme activity was expressed through Bodansky units, which represented the difference between the inorganic phosphate content of the incubated and control samples.

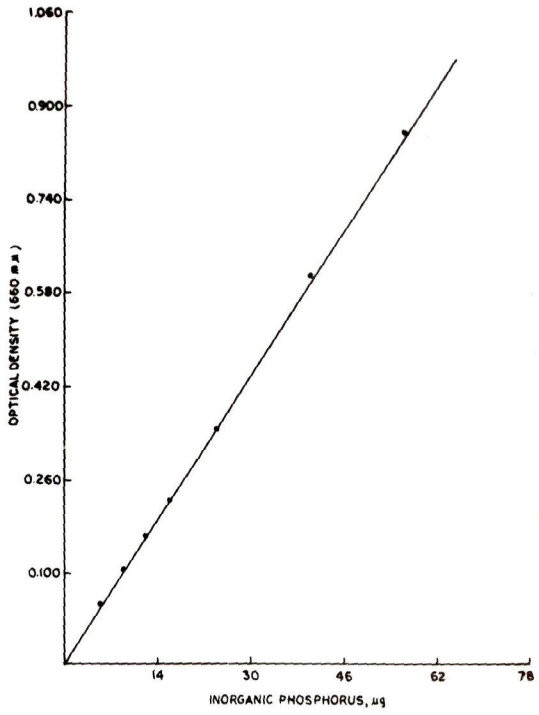
The procedure for the determination of acid phosphatase was the same as for the alkaline phosphatase, except that the buffered acid phosphatase substrate (pH 5) of Shinowara et al., as suggested by Hawk et al. (1954) was used. The calculation of results was the same as for the alkaline phosphatase.

The amino acids selected for studying their inhibitory effects were L-phenylalanine, L-methionine, L-arginine and L-valine. Known quantities of these amino acids were dissolved separately in the β-glycerophosphate substrate and the combination was warmed at 37°C. After carrying out some preliminary experiments with different concentrations (5-15 mM) of the amino acids, the final concentration of 10 mM was used for the inhibition studies.

5'-NUCLEOTIDASE ACTIVITY

For the determination of 5'-nucleotidase activity, a 10% homogenate of tissue was prepared using ice-cold distilled water as the diluent. After centrifugation at 3,000 rpm, 1.0 ml of the supernatant was used for the assay. The activity of 5'-nucleotidase was estimated by measuring the quantity of

Fig. 6. Standard curve of PHOSPHORUS.


1.060
0.900
0.740
0.580
0.420
0.260
0.100
OPTICAL DENSITY (660 mμ)
14
30
46
62
78
INORGANIC PHOSPHORUS, μg

inorganic phosphorus (pi) released from the adenosine-5'-monophosphate (AMP). The total volume of the mixture of 2.0 ml was made to contain 3 µ moles of AMP, 2 µ moles of Mg^{++} and 50 µ moles of tris-hydroxide-amino-methane-HCl (Tris-HCl) buffer of pH 8, and 0.5 ml of the tissue homogenate. The combination was incubated for 1 hour at 37°C and the reaction was thereafter stopped by the addition of an equal volume of 10% TCA. After centrifugation an aliquot of the supernatant was taken for the determination of inorganic phosphorus, using the method of Fiske and SubbaRow (1925). For reading the values of pi, a calibration curve (Fig. 6) was prepared by taking readings of a series of dilutions of standard phosphate solution of mono-potassium phosphate. The enzyme activity was expressed as µg of inorganic phosphorus liberated per ml of enzyme solution per hour.

RIBOSE NUCLEIC ACID (RNA)

Method for the extraction of RNA from the tissues and its determination by the Orcinol reaction was the same as given by Schneider (1957). The various details are as follows:

(1) Removal of Acid-soluble Compounds:

Two milliliter of a 20% tissue homogenate was mixed with 5.0 ml of cold 10% TCA and centrifuged for 10 minutes at 3,000 rpm. The supernatant was discarded and the sediment was washed with 5.0 ml of cold 10% TCA.

(2) Removal of lipoidal compounds:

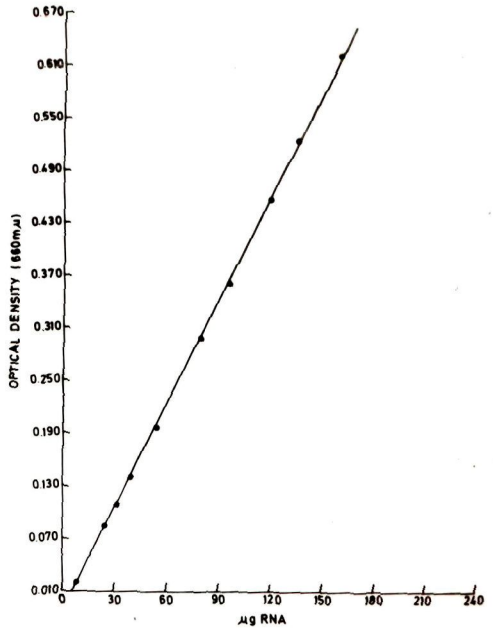
The tissue residue from (1) above was treated with 10 ml

of 95% ethanol, centrifuged and the supernatant was discarded. This operation was repeated to ensure a complete removal of phosphorus containing lipids.

(3) Removal of nucleic acids:

The pellet remaining after the removal of acid-soluble and lipoidal compounds was suspended in 2.0 ml of 1N potassium hydroxide and incubated at 37°C for 20 hours, after which 0.4 ml of 6N - hydrochloric acid and 2.0 ml of 5% TCA were added. The contents were mixed and centrifuged at 4,000 rpm for 15 minutes. The supernatant was taken for the determination of RNA by the Orcinol reaction. Orcinol was purified by dissolving it in benzene at boiling temperature (80°C) and crystallizing after adding hexane. By this treatment a white crystalline product was obtained. One gram of purified orcinol was dissolved, immediately before use, in 100 ml of concentrated hydrochloric acid containing 0.5 g of ferric chloride. A known volume (1.0 ml for muscle and 0.25 ml for liver) of the aliquot of RNA extract was raised to a volume of 2.0 ml with distilled water and an equal quantity (2.0 ml) of the orcinol reagent was added, mixed, and the samples were placed in a boiling water-bath for 40 minutes. A blank was also run by taking 2.0 ml of distilled water and 2.0 ml of the orcinol reagent. The intensity of the green color of the test solution was read at 660 mμ wave length on a Bausch and Lomb Spectronic 20 Spectrophotometer, after the instrument was set to zero density with the blank.

Fig. 7. Standard curve of RIBOSE NUCLEIC ACID (RNA).

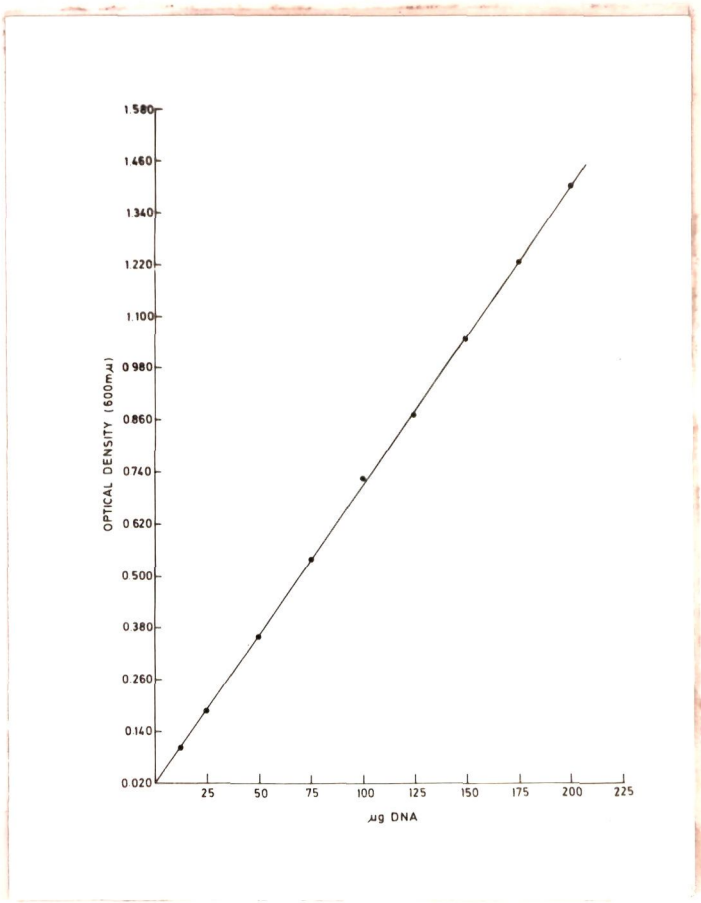

OPTICAL DENSITY (660mμ)
0.670
0.610
0.550
0.490
0.430
0.370
0.310
0.250
0.190
0.130
0.070
0.010
0
30
60
90
120
150
180
210
240
μg RNA

A calibration curve was prepared, relating the optical density to micrograms of RNA, with processed yeast RNA as the standard (Fig. 7). The results were reported as µg RNA/100 mg fresh tissue or as µg RNA-phosphorus (RNA-P)/100 mg fresh tissue on assuming that the phosphorus content of RNA is about 10% (Leslie, 1955).

DEOXYRIBOSE NUCLEIC ACID (DNA)

DNA was extracted from the tissue samples by the method of Webb and Levy (1955). Following the dehydration of the tissue residue by ether and drying in electric oven, the sample in the powdered form representing 0.5 g of the original fresh tissue was suspended in 2.5 ml of 5% TCA in pyrex glass tubes. The contents were heated in a boiling water-bath for 30 minutes, after which the tubes were removed to a cold-water bath. When the cooling was complete the volume of the contents of each tube was raised to 5.0 ml with 5% TCA. The contents were centrifuged at 4,000 rpm for 15 minutes. The supernatant was used for the determination of DNA by Croft and Lubrano (1965) modification of Burton's (1956) method of diphenylamine reaction. Analytical grade diphenylamine was purified, first by recrystallising from boiling hexane and thereafter carrying out the same treatment using petroleum ether as a substitute to hexane. A perfectly white crystalline product was obtained by this procedure. Two grames of purified diphenylamine was dissolved in 100 ml of reagent grade glacial acetic acid, and 1.5 ml of concentrated

Fig. 8. Standard curve of DEOXYRIBOSE NUCLEIC ACID (DNA).


OPTICAL DENSITY (600mμ)
1.580
1.460
1.340
1.220
1.100
0.980
0.860
0.740
0.620
0.500
0.380
0.260
0.140
0.020
25
50
75
100
125
150
175
200
225
μg DNA

analytical sulphuric acid was added. Just before use 1.0 ml of aqueous acetaldehyde (1.6%) per 20 ml of the reagent was added. One millilitre of the nucleic acid extract was mixed with two volumes of the diphenylamine reagent and the samples incubated for 45 hours at 8 ± 2°C. A blank was also run by substituting 1.0 ml of 5% TCA for the nucleic acid extract. The color intensity was read on a Bausch and Lomb Spectronic 20 Spectrophotometer at 600 mμ wave length. The value were compared with a standard curve prepared by relating the optical density to micrograms of DNA, with highly polymerized calf-thymus DNA as the standard (Fig. 3).

NITROGEN EXCRETION

The probable nitrogen excretion rates for the cat-fish, Heteropneustes fossilis were determined according to the method followed by Niimi (1972). In this method the protein losses were divided by a factor 6.25 to get the loss of nitrogen from the body. It was assumed that the entire nitrogenous fraction of the protein utilised was excreted.

DISSOLVED OXYGEN CONCENTRATION IN AQUARIUM WATER

The standard Winkler method was followed for the determination of dissolved oxygen concentration in the aquarium water.

Water sample (250 ml) was collected in a bottle and to this was added 1.0 ml of manganous sulphate solution. This was followed by the addition of 1.0 ml of alkaline potassium

iodide solution. The bottle was stoppered and the contents mixed by inverting it several times. A precipitate which appeared by this treatment was allowed to settle halfway, after which 1.0 ml of concentrated sulphuric acid was added and mixed. This dissolved the precipitate completely. After allowing the solution to stand for five minutes, a 100 ml of this solution was withdrawn into a flask and immediately titrated with 0.025 N sodium thiosulphate solution, till the yellow color of the solution almost disappeared. One milliliter of starch solution was added which developed a blue-color in the solution. The solution was again titrated with 0.025 N sodium thiosulphate solution till the blue color just disappeared (any return of blue color on standing was disregarded, as recommended). The total volume of sodium thiosulphate used in both the titrations was multiplied by 2 to get the value of dissolved oxygen in parts per million (ppm).

METHODS OF CALCULATION

(I) Regression analysis For the establishment of relationships between any two parameters (x and y) the following standard regression equation was used:

$$\text{Log } x = \text{Log } a + b \log Y$$

where, log a was the intercept and b was the slope, of the regression line. The values of log a and b were determined by the methods of least squares (Snedecor, 1959).

(II) Methods for the evaluation of Standard Deviation, Standard Error, Coefficient of Variation, Variance, Correlation Coefficient and the Standard Error of Correlation Coefficient were the same as given by Snedecor (1959). The Statistical Significance of the difference was tested through Student's t-test.

(III) Myosomatic Index: The myosomatic index was computed as myotome thickness as a percentage of total body length of fish.

(IV) Absolute growth: The absolute growth of the myotomes, in thickness was estimated as the mean thickness at each age. This has been plotted as the regression of myotome-thickness on age.

The rate of absolute growth in the thickness of myotomes has been described as yearly grains in myotome thickness per year.

(V) Relative growth: The relative growth in the thickness of myotome was calculated as an increase in growth in each time interval as a percentage of growth at the beginning of time interval.

CHAPTER I

SOME ASPECTS OF THE ANATOMY OF AXIAL MUSCULATURE OF THE FRESHWATER TELEOST *CHICHLIDALUS PUNCTATUS* BLOCH.

INTRODUCTION

Information on the details of the organisation and differentiation of lateral muscles of the trunk and tail regions, and the statistical evaluation of the various relationships, as they exist, between the dimensions of different myomeric components and the body length of the fish, especially of the Indian freshwater teleosts, seems lacking. The present chapter describes the gross morphology of a common murrel, *Ophicephalus punctatus* Bloch. Some aspects of the quantitative myology of this species have been dealt in detail.

MATERIALS AND METHODS

Ophicephalus punctatus of the size-range 120 to 248 mm were selected for these investigations.

The methods used for the measurements of various myomeric components and for establishing the statistical relationships between different myomeric components and with the body length of the fish were the same as described under 'Procedure and Methodology' (Pages 5-33).

Fig. 9. Fillet of *O. nuntalus*

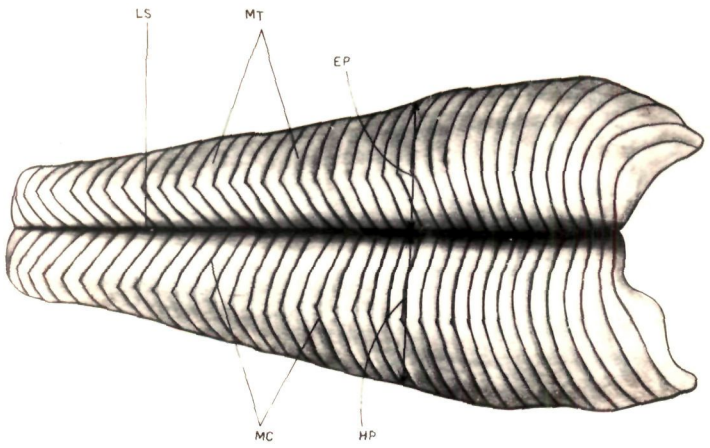
EP, epaxial portion,

HP, hypaxial portion,

LS, lateral septum,

MT, myotomes,

MC, myocommata.


LS
MT
EP
MC
HP

RESULTS AND DISCUSSION

The longitudinal somatic muscles of the trunk and tail regions were found to constitute the axial musculature of O. punctatus. The axial muscles showed perfect metamerism, being composed of a series of segments, the myotomes, which were separated from one another by myocommata (Fig. 9). The myocommata seemed to extend inward, getting attached to the vertebral column.

Architecturally, the somatic musculature of the trunk and tail of O. punctatus was typically of 'piscine' type. Each such piscine myotome of the fish was $\leq$-shaped, with markedly sharp flexures. The flexures of the myotomes formed cones with their open ends directing backward and the apices directing forward. The open ends and the apices of the flexures, on the other hand, exhibited a reverse arrangement.

The myotomes were differentiated into dorsal epaxial and ventral hypaxial portions. This division of the myotomes was brought about by the presence of a lateral septum, running along the trunk and tail regions, at the level of the lateral line (Fig. 9).

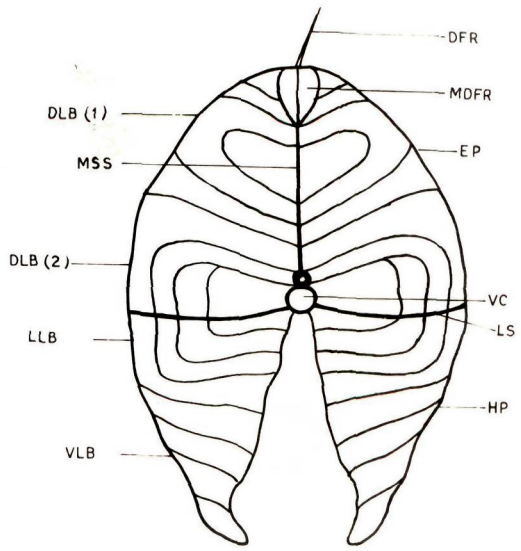
The epaxial portions of each of the myotomes of right and left sides were separated dorsally by a dorsal septum while the hypaxial portions were separated ventrally by another septum, the linea alba.

The sizes of the epaxial and hypaxial portions of each of the myotomes, as measured along the outer surface, from dorsal septum to the lateral septum and from the lateral septum to the linea alba, respectively, were found to differ markedly in O. punctatus (Table 1). The differences seemed to be due to the location of the lateral septum. Since the lateral septum was not situated exactly equidistant from the dorsal septum and the linea alba but was slightly more towards the dorsal septum, it rendered the two major muscle masses (epaxial and hypaxial) of unequal sizes. The measurements of the lengths of epaxial and hypaxial portions in fishes of the various sizes have been presented in Table 1. Myotomic measurements are given in Table 2.

In the more anterior region of the fillet, the lateral septum was found to take a curve. This curve was convex towards the hypaxial region and was then found to lift up markedly towards the epaxial region, with the result the hypaxial region, at the point where the curve attained its maximum convexity, was rendered shorter than the region lying anterior to it, from where the lateral septum was lifted upwards. On the contrary, the condition was just the reverse for the epaxial region. The corresponding epaxial region, where the lateral septum reached its maximum concavity was of greater size than the region from where the lateral septum was lifted up. This disparity in the sizes was marked with respect to either the epaxial or the hypaxial regions and in no case, however, the hypaxial portion

Fig. 10. Cross section of *C. punctatus* through the region of great girth, adjacent to dorsal fin.

EP, epaxial portion,
HP, hypaxial portion,
LS, lateral septum,
MLB(1), first dorsal longitudinal bundle,
MLB(2), second dorsal longitudinal bundle,
LLB, lateral longitudinal bundle,
VLB, ventral longitudinal bundle,
MDFR, musculature of dorsal fin ray,
DFR, dorsal fin ray,
VC, vertebral centrum,
MSS, median skeletogenous septum.


DFR
MDFR
DLB (1)
EP
MSS
DLB (2)
VC
LS
LLB
HP
VLB

was shorter than the corresponding epaxial portion of the same myotome (Fig. 9).

The lateral septum was found to extend inward, meeting the centra of the vertebrae. The measurements of the distance which it so traversed, in specimens of the various sizes investigated have been given in Table 1 .

The muscle segments of the lateral musculature of either side were found to extend from below the skin deep into the body axis and became limited at the midline by a median skeletogenous septum (Fig. 1c).

The lateral septum which separated the two major muscle masses of either side by its very location, and the median skeletogenous septum by its vertical position, resulted in the arrangement of the trunk and tail muscles into quadrants. The epaxial muscles were found to constitute the upper or dorsal quadrants while the hypaxial muscles constituted the lower or ventral quadrants. The epaxial portions of the myotomes of C. punctatus were composed of two dorsal longitudinal bundles while the hypaxial muscles were composed of a lateral and a ventral longitudinal bundles (Fig. 1c).

It was observed that in extending inward, the myotomes and the myocommata did not meet the sagittal axis of the body at right angles but intercepted it at an angle which was more

Fig. 11. Relationship between the length of epaxial portion and body length of *O. punctatus*.

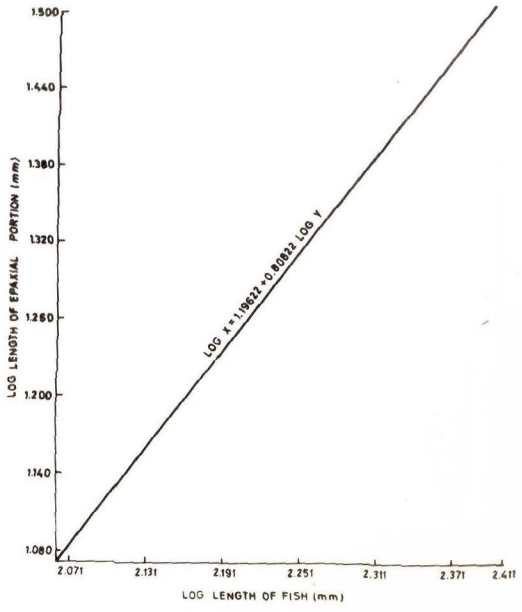
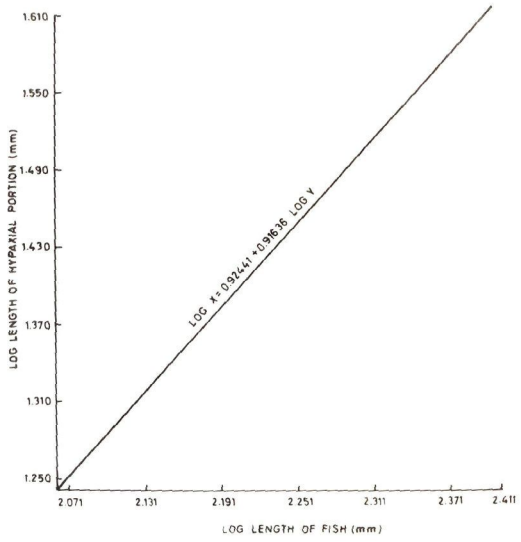

1.500
1.440
1.380
1.320
1.260
1.200
1.140
1.080
LOG LENGTH OF EPAXIAL PORTION (mm)
LOG X = 1.18622 + 0.80822 LOG Y
2.071
2.131
2.191
2.251
2.311
2.371
2.411
LOG LENGTH OF FISH (mm)

Fig. 12. Relationship between the length of hyparial portion and body length of *Q. punctatus*.


1.610
1.550
1.490
1.430
1.370
1.310
1.250
LOG LENGTH OF HYPAXIAL PORTION (mm)
LOG X = 0.92441 + 0.91636 LOG Y
2.071
2.131
2.191
2.251
2.311
2.371
2.411
LOG LENGTH OF FISH (mm)

sharp in the region of the tail. This became evident when a needle was inserted at right angles to the sagittal plane in a particular myotome of the tail region: the needle passed this myotome and entered the other myotome instead of going all the way into the same myotome, indicating that the myotomes did not extend at right angles to the sagittal axis. A somewhat similar pattern of arrangement of the myotomes and myocommata has been reported by Love (1970) in the cod, Gadus morhua, where these were found not to run vertically from 'bone' side to 'skin'/side but taking a curve towards the tail and within the thickn of the fillet in a rather complex pattern.

The thickness of the myotomes and the lengths of the epaxial and hypaxial portions were found to be in correlation with the body length of the fish (Figs.11,12,14,15). The relationship between the thickness of the myotomes and the body length of O. mykiss has been discussed in a separate chapter (pages 41-46).

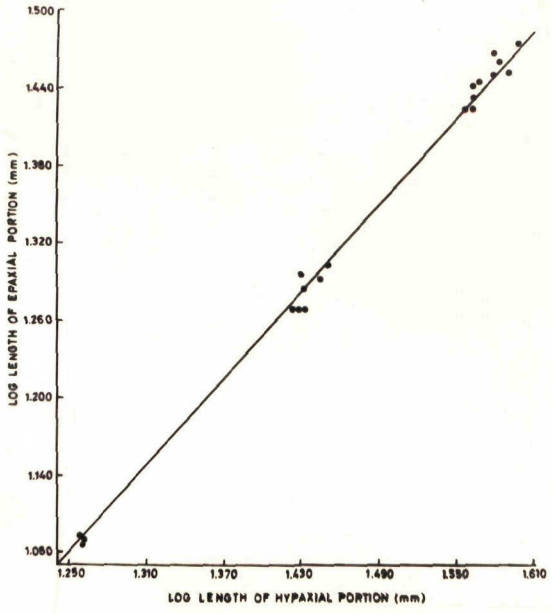
The relationship between the lengths of the epaxial and hypaxial portions with the body length of the fish as worked out through regression methods could be expressed as:

$$\text{Log } x = 1.1962 + 0.8082 \log y$$

Where, x was the body length of the fish (mm), and y was the length of the epaxial portion (mm).

$$\text{Log } x = 0.9244 + 0.9163 \log y$$

Fig. 15. Hyparial/eparial length relationship in *O. punctatus*.


1.500
1.440
1.380
1.320
1.260
1.200
1.140
1.080
LOG LENGTH OF EPAXIAL PORTION (mm)
1.250
1.310
1.370
1.430
1.490
1.550
1.610
LOG LENGTH OF HYPAXIAL PORTION (mm)

Where, x was the body length of the fish (mm), and y was the length of the hypaxial portion (mm).

The logarithmic equation establishing the relationship between the lengths of the epaxial and hypaxial portions was worked out to be:

$$\text{Log } x = 0.2776 + 0.8964 \log y$$

Where, x was the length of the hypaxial portion (mm) and y was the length of the epaxial portion.

The above relationship between the hypaxial and epaxial portions has been graphically shown (Fig. 13).

The correlation coefficients between the body length and the length of the epaxial portion was found to be 0.9936, significant at 0.001 level of probability and that between the body length and the length of the hypaxial portion was 0.9930, also significant at 0.001 P. The correlation between the lengths of epaxial and hypaxial portions was also statistically significant ($P < 0.001$).

SUMMARY

The lateral (axial) musculature of the trunk and tail regions of O. punctatus was found typically of 'piscine' type. In extending inward, the myotomes and myocommata did not meet the sagittal axis at right angles. The logarithms of the lengths

of each of the epaxial and hypaxial portions of the myotomes maintained direct proportionality with the logarithms of the body length of the fish. The statistical analysis of the intraspecific epaxial and hypaxial length relationship gave 0.2776 and 0.8964 regression constants. The correlation coefficient between the epaxial length and body length, and that between hypaxial length and body length were found to be significantly higher ($P < 0.001$).

CHAPTER II

GROWTH CHARACTERISTICS OF THE MYOTOMES OF *OPHICEPHALUS PUNCTATUS* BLOCH.

INTRODUCTION

Despite considerable work on the growth and development of various anatomical structures in fishes (Battle, 1944; Mahon and Hoar, 1956; Trinkaus and Wake, 1956; Armstrong and Child, 1965; Daniel *et al.*, 1966; Dickson and Battle, 1970; Morerosa *et al.*, 1974; Kuruna and Ivano, 1974) information on the growth of the myotomes, hitherto, remains inadequate. The present chapter describes the growth characteristics of the myotomes from juvenile to adult stages of *Ophicephalus punctatus* Bloch. The quantitative analysis of the growth of myotomes, in the present investigation, consists of fitting straightline logarithmic plots between myotome thickness and body length of the fish, to regression formulae, besides establishing the age-specific pattern of the growth of an anterior and a posterior myotome.

MATERIAL AND METHODS

Specimens of the size-range 120 to 240 mm, belonging to year-classes 0^+ to 3^+ were obtained from the local ponds of Aligarh. The recognition of the different age-groups was based on the examination of scales. The thickness of 4th and 21st

Fig. 14. Relationship between the thickness of 4th myotome and body length of *O. punctatus*.

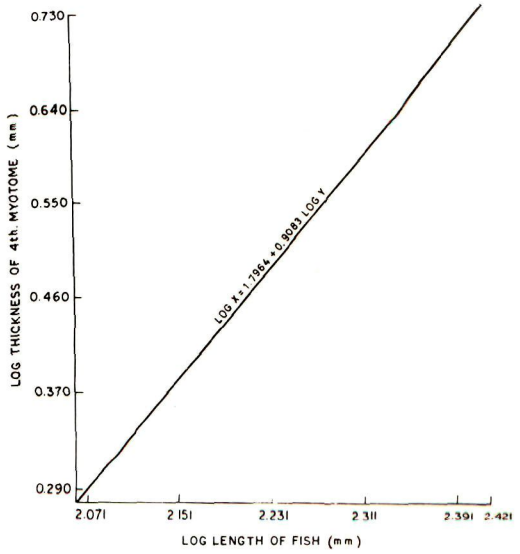
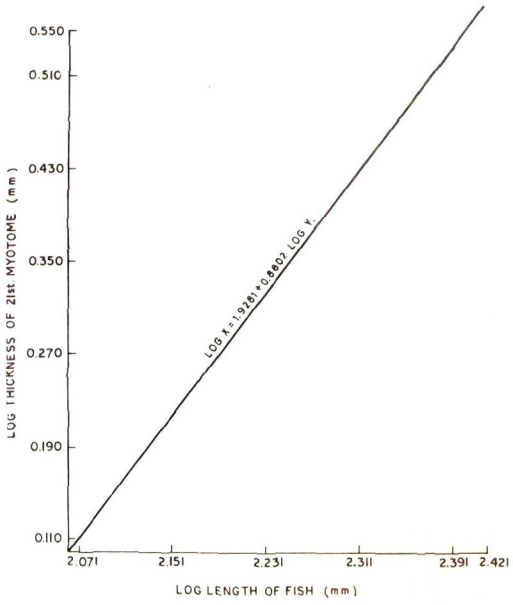

0.730
0.640
0.550
0.460
0.370
0.290
LOG THICKNESS OF 4th MYOTOME (mm)
LOG X = 1.7864 + 0.8083 LOG Y
2.071
2.151
2.231
2.311
2.391
2.421
LOG LENGTH OF FISH (mm)

Fig. 15. Relationship between the thickness of 21st myotome and body length of *O. punctatus*.


0.550
0.510
0.430
0.350
0.270
0.190
0.110
LOG THICKNESS OF 21st MYOTOME (mm)
LOG Y = 1.9281 + 0.8802 LOG X
2.071
2.151
2.231
2.311
2.391
2.421
LOG LENGTH OF FISH (mm)

myotomes, absolute and relative growths, myosomatic indices, together with the intraspecific variations were assessed by the methods mentioned under 'Procedure and Methodology' (pages 9-33)

RESULTS

A direct correlation was found to exist between the growth of the myotomes in thickness and the total body length of the fish. The logarithmic transformation of the 4th myotome thickness - body length relationship was expressed by the equation:

$$\text{Log } x = 1.7964 + 0.9083 \log y$$

where, x was the body length of the fish (mm) and y was the thickness of the 4th myotome (mm). The correlation Coefficient, r (0.997±0.0011) was found to be significant at 0.001 level of probability. The equation establishing the relationship between the 21st myotome - thickness and body length of the fish was found to be:

$$\text{Log } x = 1.9281 + 0.8802 \log y$$

where, x was the body length of the fish (mm), and y was the thickness of 21st myotome (mm). The correlation coefficient, r (0.998±0.0045) was significantly high ($P < 0.001$).

These relationships have been shown graphically (Figs. 14, 15).

Fig. 16. Correlation between the growth of the 4th myotome and that of the body length of O. punctatus.

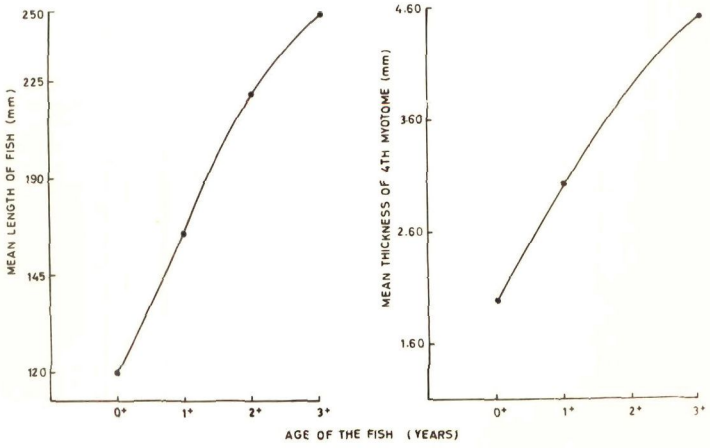

250
225
190
145
120
MEAN LENGTH OF FISH (mm)
0+
1+
2+
3+
4.60
3.60
2.60
1.60
MEAN THICKNESS OF 4TH MYOTOME (mm)
0+
1+
2+
3+
AGE OF THE FISH (YEARS)

Fig. 17. Differential growth characteristics of the myotomes of *O. punctatus*.

A, absolute growth of 4th myotome.

B, absolute growth of 21st myotome.

C, growth rate of 4th myotome.

D, growth rate of 21st myotome.

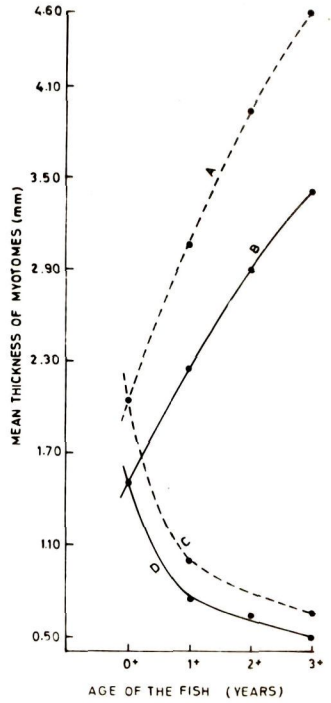
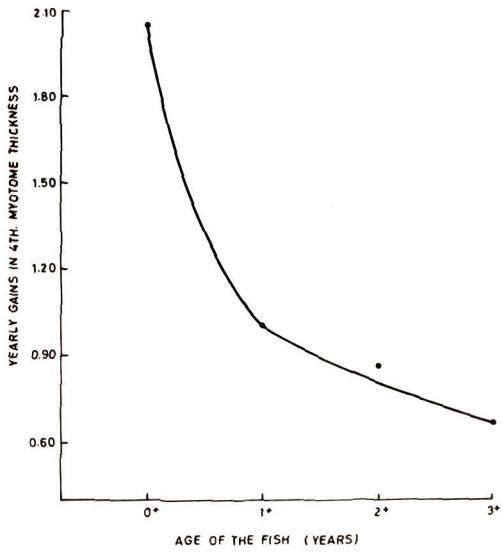

4.60
4.10
3.50
2.90
2.30
1.70
1.10
0.50
MEAN THICKNESS OF MYOTOMES (mm)
A
B
C
D
0+
1+
2+
3+
AGE OF THE FISH (YEARS)

Fig. 16. Yearly gains in the thickness of 4th myotome of *O. punctatus*.

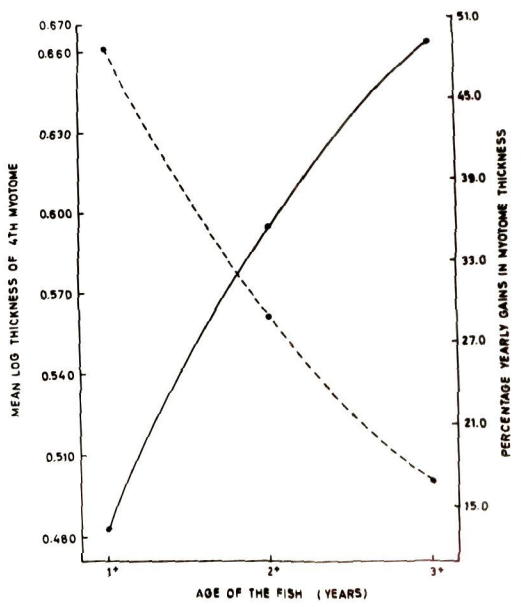

2.10
1.80
1.50
1.20
0.90
0.60
YEARLY GAINS IN 4TH. MYOTOME THICKNESS
0+
1+
2+
3+
AGE OF THE FISH (YEARS)

The myotome thickness - body length relationship was also expressed by myosomatic index. The myosomatic index (for 4th myotome and body length) ranged from 1.700 to 1.846 (Table 3 pertaining to 21st myotome, however, the myosomatic index varied from 1.250 to 1.366 (Table 3).

The present study conducted on various year-classes of L. punctatus revealed that different life periods of the fish were characterized by different characters of myotomic growth, i.e., the growth of the myotomes was age-specific, being rapid during the first year of life and declining thereafter. Strikingly enough, the growth in the body length of this species was also recorded to be rapid during the first year of life, but slowed down progressively during the subsequent years (Fig. 16). The absolute growth of the myotomes indicated an increasing slope in the beginning, followed by a decreasing slope (Fig. 16). The myotomes continued to increase in thickness as a result of absolute growth, but the rates of growth declined considerably with age. This decline in the growth rate of the myotomes was also evident when yearly gains plotted against time gave declining curves (Figs. , 18).

The relative growth of the myotomes, when plotted as the logarithms of myotome thickness against time, produced a curve which was found to rise steeply in the beginning and declining progressively thereafter. When the percentage yearly gains were

Fig. 19. Relative growth of 4th myotome of *O. punctatus*.


0.670
0.660
0.630
0.600
0.570
0.540
0.510
0.480
MEAN LOG THICKNESS OF 4TH MYOTOME
51.0
45.0
39.0
33.0
27.0
21.0
15.0
PERCENTAGE YEARLY GAINS IN MYOTOME THICKNESS
1+
2+
3+
AGE OF THE FISH (YEARS)

plotted against time, however, a continuous decline in the relative growth curve became obvious (Fig. 19). The percentage yearly gains at each age and the mean thickness of the myotome in *L. punctatus* of each year-class have been shown in Table 4 .

Another interesting fact that has emerged from the present investigation was that each myotome of fish maintained its own rate of growth. In *L. punctatus* the growth rate of 4th myotome was found to be comparatively faster than that of the 21st myotome (Fig.17). The ratios of these differential growth rates varied within a narrow range. For the fishes of 0^+, 1^+, 2^+ and 3^+ year-classes, these ratios were found to be 1.360, 1.351, 1.353 and 1.323, respectively. The analysis of intraspecific variations in these ratios gave a standard deviation of 0.0177. The variance and the coefficient of variation being 0.0003 and 1.3137, respectively. These ratios and their intraspecific variations have been given in Table 4 .

DISCUSSION

Concordant to the observation of Love (1958), the regularit with which the myotomes of *L. punctatus* were found to grow in thickness was accompanied by an increase in the body length of the fish. The proportionality between the growth of the body and the myotomes was apparent from the constancy as exhibited by the myosomatic indices which did not allow any marked divergence from the linear relation.

The decline in the absolute and the relative growth of the myotomes was found to be the function of increasing age. The disparity between the absolute and the relative growth of the myotomes was more marked during the younger than during the older ages, as in the older year-classes the growth of the myotomes was slow and the differences in the growth were comparatively little, both from the absolute as well as the relative view point. As mentioned above, the myotomes differed in their rates of growth but the ratio of their growth rates varied only within a very restricted range. Presumably, it was this constancy in the ratios of the growth rates of myotomes which was responsible for maintaining a definite differential in their thickness.

It was evident that if the normal proportions of the myotomes were to be maintained, the constancy in the ratios of the differential growth rates, besides the one in the myosomatic indices, was a prerequisite. The narrow range of intraspecific variations, however, seemed to lack the potential of giving unusual dimensions to the myotomes of the fish.

SUMMARY

A positive correlation was found to exist between the growth of the myotomes and that of the body length of L. punctatus. The changes in the absolute and the relative growth of the myotomes, as observed in this fish, were found to be age-specific. The growth rate of the 4th myotome was faster than that of the

21st myotome. The constancy in the myosomatic indices as also in the ratios of the differential growth rates of the myotomes did not seem to permit any marked divergence from the linear relation between the growth of the body and the myotomes.

CHAPTER III

VARIATIONS IN SOME CHEMICAL CONSTITUENTS OF THE TRUNK AND TAIL SECTIONS OF THE BODY MUSCULATURE OF *OPHICEPHALUS PUNCTATUS* BLOCH.

INTRODUCTION

Although literature reporting variations in the biochemical composition of the edible portion of fish flesh in relation to size, sex, season, stage of maturity, catchment locality, as reviewed by Love (1970), is fairly extensive, there seem to be relatively fewer accounts dealing with the differences in the chemical composition of different parts of the fillet of an individual fish (Matsumoto, 1950; Brandes and Dietrich, 1953a; Hirao *et al.*, 1954a,b; Higashi *et al.*, 1957, 1958; Kanemitsu and Aoe, 1958; Love, 1958b; Olley and Lovern, 1960; Hannan *et al.*, 1961a,b; Karrick and Thurston, 1964; Anon, 1966a; Koizumi *et al.*, 1967; Jafri, 1973). The present account is based on the quantitative estimation of principal chemical constituents, total carbohydrates, glycogen, ribose nucleic acid (RNA) and deoxyribose nucleic acid (DNA), cholesterol and energy value in terms of calories, in the trunk and tail sections of the fillet of *Ophicephalus punctatus* Bloch.

MATERIALS AND METHODS

Methods employed for the procurement of *O. punctatus* and their maintenance in the laboratory aquaria, tissue sampling and

the details of the analytical procedures, and quantitative evaluation of the data were the same as described under 'Procedure and Methodology' (pages 9-33).

RESULTS

The concentrations of various chemical constituents and energy value of the trunk and tail sections of the fillet of Ophiocephalus punctatus Bloch. have been presented in Table It would be evident from the data that the principal chemical constituents (protein, fat, water, ash), total carbohydrates, glycogen, RNA, DNA and cholesterol were not distributed homogeneously in the body musculature. With the exception of water, whose distributional characteristic was in sharp contrast to the fat, all the constituents were more concentrated in the muscles of the tail end of the fillet. The differences, though less marked for some chemical constituents, were significant for the others. With the higher levels of energy substances, including protein, fat and carbohydrate, the tail section was obviously more rich in calorific value.

DISCUSSION

The coincidence in the pattern of progression in the levels of both protein and RNA revealed the importance of this nucleic acid in protein synthesis. The rise in the RNA and protein concentrations towards the tail end of the musculature

have been reported by Edstrom (1964), and Brandes and Dietrich (1953a), respectively. The positive quantitative correlations between RNA and protein have been reviewed by Brachet (1955) in different anatomical structures.

The progressive decline in the thickness of myotomes towards the tail end of the musculature (Chapter I) resulted in a close set-up of the RNA rich connective tissue septa (myocommata) apparently increased the RNA concentration. A corollary to this finding is evident from the work of Love (1958). In addition to this the slower growth rates of the posteriorly located myotomes (Chapter II) and hence of the muscle cells contained in them, caused a larger number of cells to be sampled in a unit weight of the muscle tissue. This can also account for higher RNA concentration of the tail muscles since the value of RNA in a tissue is directly related to the number of cells present in it (Hotchkiss, 1955; Leslie, 1955; Bulow, 1970).

Higher concentration of fat observed in the tail region of the fish appeared to correspond with the concentration of myocommata in these regions, which, as reported by Love (1970), contain higher levels of fat than the myotomes.

The decline in the values of water towards the tail end of the musculature was the result of its reciprocal correlation with the fat.

Greater concentration of ash in the tail section of the fish was perhaps due to a greater concentration of some of the major inorganic substances. An analysis of different regions of fish fillet carried out by Thurston and Maclaster (1960) and Thurston (1962) also indicated to an increase in substances, like sodium and potassium, at the tail end. Kruchkova (1952) correlated this increase in the concentration of mineral substances towards the tail end with an increase in the metabolic activity of the muscles. Some other biochemical and physiological investigations, however, did not indicate to higher metabolic activity of the tail muscles. The studies on the distribution of energy rich phosphate compounds in different sections of fish musculature revealed no preferential accumulation of these substances in any one part (Nagayama, 1961a). The observations of Ammo et al. (1953), reporting lowest pH as a consequence of considerable accumulation of lactic acid, through the breakdown of glycogen, in the mid- and not the tail-section provided no indication of any greater physiological role of the muscles at the tail region. This was supported by subsequent investigations by Black et al. (1962), documenting a decline in the lactic acid to glycogen ratio towards the tail end after muscular activity.

It can be concluded that the observed increase in the concentrations of total carbohydrates, glycogen, cholesterol, and the various other constituents, with the exception of water,

towards the tail region of the musculature was due to an increase in the number of cells per unit weight, as also in the proportion of connective tissues, rather than in the metabolic activity. This conclusion was in agreement with the one arrived at by Love (1970) for some chemical constituents of fish.

The differences in the concentrations of various chemical constituents in different regions of the musculature of *L. punctatus*, in addition to signifying the variations in the nutritive value of different sections of fillet, also impress upon the need to select some particular myotomes for analysis, preferably by dissecting them free from the myocommata, to eliminate the sources of error related to the anatomical specificity in the levels of the chemical constituents.

SUMMARY

Distribution of some chemical constituents in the trunk and tail sections of the fillet of *Ophicephalus punctatus* Bloch. was described. Chemically, the musculature was found to be fairly heterogeneous. The increase in the levels of the principal chemical constituents, carbohydrates, glycogen, RNA, DNA and cholesterol was the result of increases in the number of cells per unit weight of the sample and in the concentration of myocommata. The distribution of water in the musculature was inversely related to that of fat. The synthesis of protein was found to be related to the level of RNA.

CHAPTER IV

SOME BIOCHEMICAL CONSTITUENTS OF THE DARK AND WHITE MUSCLES AND THE LIVER OF THE CAT-FISH, CLARIAS MAGUR (LINN.)

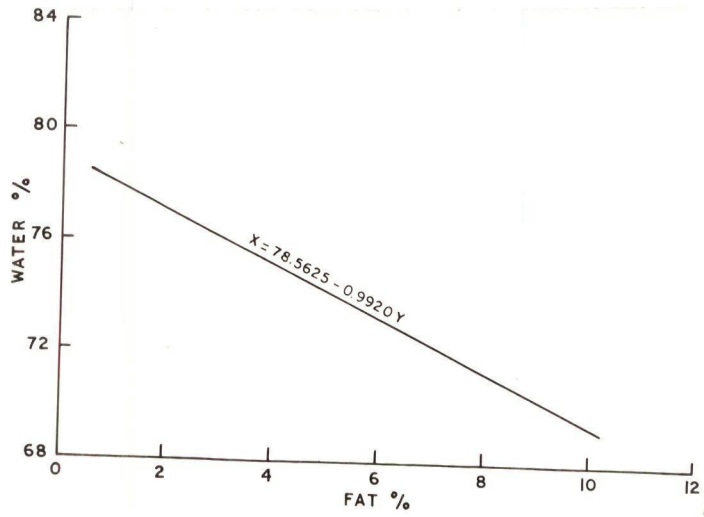
INTRODUCTION

This chapter presents information on the distribution of proximate chemical constituents, total carbohydrates, glycogen, cholesterol and nucleic acids in the dark, white muscles and liver of Clarias magur (Linn.), a freshwater cat-fish. Effort has been made, where possible, to elucidate the physiological basis of the biochemical similarities or differences in the three mentioned tissues, in order to throw some light on the functional affinities of the dark muscle with the white on the one hand and with the liver on the other.

MATERIALS AND METHODS

Methods for the procurement of live specimens of Clarias magur, their maintenance in the laboratory aquaria, sampling of the tissues, and the quantitative estimations of the various chemical constituents, together with the statistical analysis of the data were the same as described under 'Procedure and Methodology' (pages 9 - 35).

Fig. 20. Fat/water relationship in *C. maxur*.


84
80
76
72
68
WATER %
X = 78.5625 - 0.9920 Y
0
2
4
6
8
10
12
FAT %

RESULTS

The concentrations of the various biochemical constituents in the dark and white muscles and the liver of *Clarias batrachus* have been given in Table 6 .

PROTEIN- Of the three tissues analysed, the white muscle was found to be richest in the protein content, followed by the dark muscle; the liver being the poorest source of this constituent.

FAT- Next to liver, the higher fat concentration seemed to occur in the dark muscle, while the white muscle being the poorest in this constituent.

WATER- Unlike the distributional characteristic of fat, the water content was observed to be highest in the white muscle, followed by the dark muscle and the liver. Such a pattern of water distribution in the three tissues, reflected a reciprocal correlation vis-a-vis fat (Fig. 20). The relationship between the two constituents could be expressed by the following equation.

$$\text{Fat (\%)} = 78.5625 - 0.9920 \text{ water (\%)}$$

The correlation coefficient (r) for this relationship was found to be -0.990, significant at 0.001 level of probability.

ASH- Among the three tissues investigated, the ash content of the dark muscle was lower than that of the white, while the liver contained the least quantity of ash.

TOTAL CARBOHYDRATES- The total carbohydrate concentration was highest in the liver, followed by the dark muscle; the white muscle was poorest in this energy constituent.

GLYCOGEN- The pattern of distribution of this polysaccharide was similar to that of the total carbohydrates with dark muscle containing greater concentration than the white, and the liver having the highest concentration.

CHOLESTEROL- The cholesterol concentration of the dark muscle appeared to be higher than that of the white, but was definitely lower than that of the liver.

NUCLEIC ACIDS- The liver was found to be the richest source of both RNA and DNA, followed by the dark muscle and the white.

DISCUSSION

A survey of the literature revealed that a similar pattern of protein distribution in the dark, white muscles and liver, as observed in the present study, has been documented by Brockman (1959) in several fish species. Although the author is aware of no other published report dealing with comparative analysis of the protein in the three tissues, quite a few studies on the biochemical constituents of the dark and white muscles, excluding the liver, are available. Excepting for the work of Alexander (1955), the other investigators (Fujikawa and Haganuma, 1936; Iyer et al., 1963) reported lower protein concentration in the

dark muscle, in comparison to the white.

With respect to fat concentration, the dark muscle resembled more to the liver. Some of the earlier observations (Fujikawa and Nagamura, 1936; Alexander, 1955; Brockhan, 1955; George, 1962; Thurston, 1962; Iyer et al., 1963; Zama, 1963a; George and Bokdawala, 1964; Ellis and Scott, 1966) also revealed that dark muscle contained considerably higher concentration of the fat than the white muscle. By virtue of its higher fat content, it was considered that, unlike the white muscle, the dark muscle was not well suited for mechanical activity. This finding has gained support from a number of other investigations (Brockhan, 1959, Tsuchiya and Kunii, 1960; Barets, 1961; Wittenberger, 1960; Wittenberger and Gros, 1961). The close proximity of the dark muscle to the white seemed an obvious advantage for the transfer of metabolites (Brockhan, 1956, 1959). Consequently, the striking location of the dark muscle along the lateral line appeared to be an advantage to provide energy from its rich fat depot to the mechanical body tissue at times of greater physical activity. Strikingly enough, the proportion of the dark muscle to the white has been reported to increase with the swimming activity of the fish; being considerably less in fishes which rest on the sea bottom than in the pelagic ones which swim continuously (Love, 1970).

The higher fat concentration in the dark muscle, in comparison to the white, also appeared to be in keeping with

its higher capacity towards lipid metabolism. From the investigations of Bilinski (1963), Bilinski and Jonas (1964), Jonas and Bilinski (1964) it was, indeed, found that the dark muscle was one of the tissues with a greater capacity towards fatty acid oxidation. Further studies carried out by George (1962) and George and Bokdawala (1964) revealed greater lipase activity in the dark muscle and pointed that this muscle was well adapted to utilise lipid as its chief fuel.

The degree of hydration in the tissues maintained an inverse progression with that of the fat.

From the relatively higher concentration of ash in the white muscle it appeared as if this mechanical tissue required greater amounts of some inorganic substances than the dark muscle, whose function, in some respects has been reported to be similar to that of an internal organ, specially the liver which contained the least concentration of ash. Previous studies, notably those of Alexander (1955), Braekkan (1959) and Iyer et al. (1963) also reported higher concentration of ash in the white muscle than the dark.

Higher concentration of cholesterol in the dark muscle in comparison to the white, as observed in the present study, could be attributed to some coincidence in the physiological roles of the dark muscle and the liver (Zaam, 1963a). Igarashi et al. (1957b) also documented higher ash level in the dark muscle.

The greater quantity of carbohydrates in the dark muscle and the liver represented a reserve for the supply of energy to the mechanical tissue at times when the calorific demands could become too high to be met at the expense of white muscle glycogen. A locational juxtaposition of the dark and white muscles was, therefore, of considerable help in providing energy for immediate needs. A higher glycogen content in the dark muscle, in comparison to the white, has also been recorded by Bmaekkan (1956), Buttkus (1963), Bone (1966), and George and Bokdawala (1964). The investigations carried out by Fraser et al. (1966), however, revealed no consistent difference in the glycogen levels of the two types of muscles.

The higher levels of RNA in the dark muscle and the liver implied greater physiological activity of these tissues. The investigations of Brachet (1955) and Leslie (1955) left no doubt that a positive correlation did exist between the RNA level and the metabolic activity of tissue. The greater concentration of RNA in the dark muscle, in comparison to that in the white, implied that the dark muscle could be an important location of a variety of metabolic functions resembling the liver.

Insofar as the level of RNA is related to the number of cells contributing to the unit weight of the tissue, a distributional characteristic of the RNA in the order: liver - dark muscle - white muscle, reflected that among the three tissues,

the cells of the liver were narrowest, contributing by far the most number of cells to the unit weight of the liver sample, while the cells of the dark muscle though smaller than those of the white might be of larger dimensions in comparison to the hepatic cells, and thus the number of cells expected to contribute to the given weight of the tissue sample was higher in the dark muscle than the white. Some of the anatomical observations (Boddeke et al., 1959; Thurston and Mackaster, 1960; George, 1962; Buttkus, 1963; George and Bokdawala, 1964, and Nishiham, 1967) have indeed reported such a difference in the cell sizes of the two muscle types. Perhaps, this was one of the main basis of the differences in the amounts of DNA found in the unit weight of the various tissues. That the amount of DNA in the given weight of the tissue was related to the number of cells present in it, has been well documented (Hotchkiss, 1955; Leslie, 1955). However, the differences in the DNA synthesis of different tissues cannot be ruled out.

SUMMARY

Distribution of some chemical constituents was studied in the dark and white muscles and the liver of Clarias mamur (Linn.). Of the three tissues, the white muscle contained the highest quantities of protein, water and ash. The liver was poorest in these constituents but appeared to be the richest source of fat, total carbohydrate, glycogen, cholesterol, RNA

and DNA. The concentrations of these constituents also seemed to be higher in the dark muscle than in the white. Water content was reciprocally related to the level of fat in the three tissues.

From the analysis of the various chemical constituents in the dark and white muscles and in the liver of C. mauri, it appears that, although differences do exist between the two types of muscles, any attempt to conclude finally, the physiological affinities of the dark muscle with either the white muscle or the liver may raise questions leading to a number of speculations. Further investigations, perhaps of a more experimental nature, must be carried out before arriving at any final conclusion.

CHAPTER V

CHANGES IN THE PROXIMATE CHEMICAL CONSTITUENTS OF THE FLESH OF THE FRESHWATER MURREL, *CHANNA PUNCTATUS* BLOCH DURING GROWTH

INTRODUCTION

Statistical descriptions of the growth of fish have been presented by many workers in the past (Gordon, 1967; Brain, 1967 Beamish, 1973; Nicholls, 1973; Richards, 1973; Westrheim, 1973; Bowers, 1974; Gunderson, 1974). The conclusion common to all is the change in size and weight. The growth, however, is a general term for a very complex change depending upon many factors from the simple imbibition of water to the complicated results of nutrition chemistry (Rounsefell and Everhart, 1953). The fact that only quantitative analysis of growth consisting of attempts to fit a number of growth curves to some formulae, tells nothing about the underlying mechanism of organic growth (Bertalanffy, 1949), in the present study on the biochemical changes characterizing the growth of a freshwater fish, the author has attached to mathematical function a rational, as opposed to a merely descriptive significance. The changes in the proximate chemical constituents occurring during growth seemed in conformity with the fact that the growth of a fish results from the consumption of food, its assimilation and incorporation, primarily, as proximate chemical constituents which construct the animal's body. After the attainment of

Fig. 21. Proximate chemical constituents (Protein, o——o ;
Fat, o - - - o ; Water, o . . . o ; Ash, o o)
in the flesh of O. striatus of different ages.

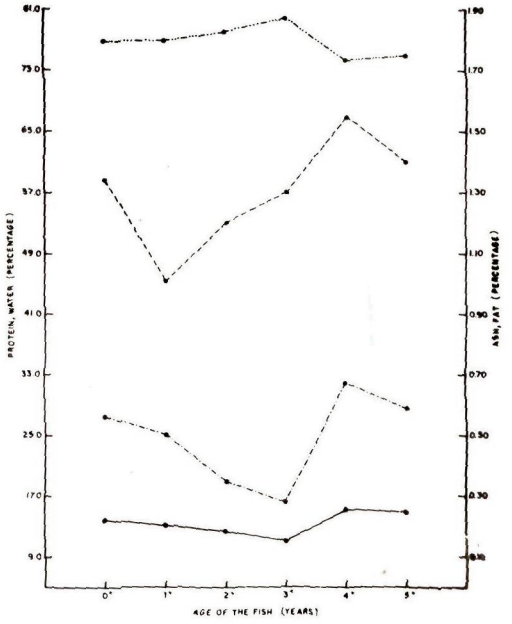
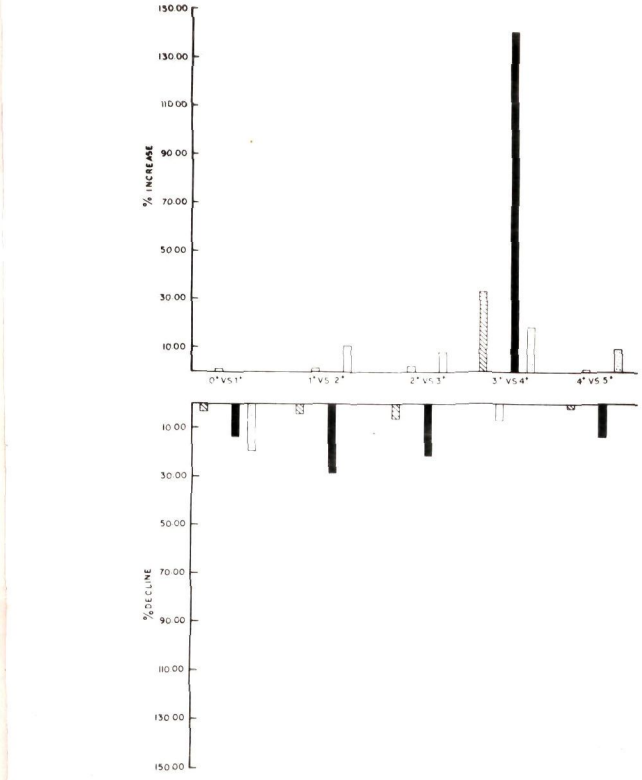

PROTEIN, WATER (PERCENTAGE)
ASH, FAT (PERCENTAGE)
AGE OF THE FISH (YEARS)
0+
1+
2+
3+
4+
5+

Fig. 22. Percentage increase and decline in protein (striped bars), fat (black bars), water (white bars) and ash (dotted bars) in *O. striatus* of different ages.


% INCREASE
150.00
130.00
110.00
90.00
70.00
50.00
30.00
10.00
0° VS 1°
1° VS 2°
2° VS 3°
3° VS 4°
4° VS 5°
% DECLINE
10.00
30.00
50.00
70.00
90.00
110.00
130.00
150.00

maturity, however, the main part of the food consumed by the fish is not used for linear growth but for the ripening of the gonads (Love, 1970).

The present study is based on the principal chemical constituents of Ophicephalus striatus, a freshwater murrel, which grows to about three feet or more in length and inhabits rivers, irrigation channels and large weedy ponds. The fish contributes to the bulk of the freshwater fish catch. High nutritive value of its flesh makes it an important food fish and impresses for the need to culture this species on scientific lines.

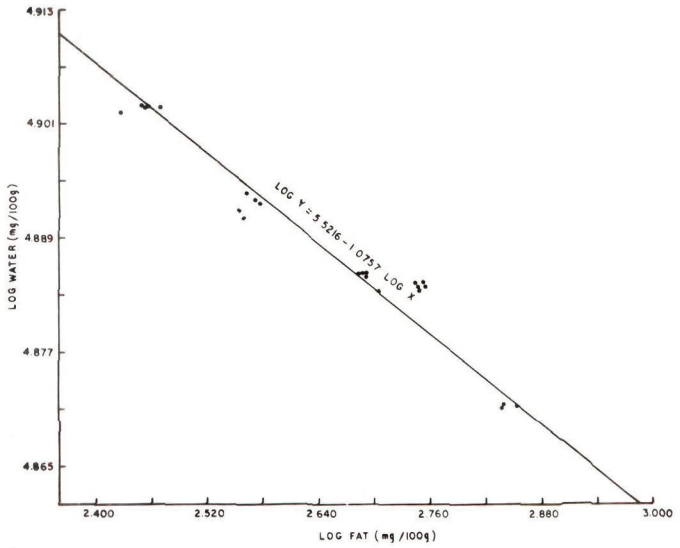
MATERIALS AND METHODS

Details of the methodology of procurement and maintenance of Ophicephalus striatus, age determination, tissue sampling, chemical analysis and the statistical interpretation of the data have been given under 'Procedure and Methodology' (pages 9-33).

RESULTS

The age specific changes in the concentrations of principal (or proximate) chemical constituents have been given in Fig. 21 and Table 7 , and percentages of their increase and decline, in the specimens of successive year classes have been represented in Fig. 22. Protein content of the muscle ranged from 11.282%

Fig. 23. Fat/water relationship in *O. striatus* of various ages.


4.913
4.901
4.889
4.877
4.865
LOG WATER (mg/100g)
LOG Y = 5.5216 - 1.0757 LOG X
2.400
2.520
2.640
2.760
2.880
3.000
LOG FAT (mg/100g)

to 15.155% in the fishes of 0⁺ - 5⁺ year-classes. The values were found to decline in the year-classes from 0⁺- 3⁺. In the fishes of 4⁺ year class, however, there seemed a distinct rise in the protein content. Further growth of the fish, however, did not seem to characterise any marked changes in the muscle protein content.

Fat was found to be the most dynamic constituent, undergoing marked changes during different periods of growth of the fish. The values of the muscle fat content in different year-classes ranged from 0.201% to 0.670%. A continuous fall in the muscle fat was noted in the fishes from 0⁺- 3⁺ year-classes. A recovery was, however, found to occur in fishes of 4⁺ year-class and thereafter the values declined slightly.

Water content of the muscle seemed to rise in the successive year-classes from 0⁺- 3⁺, but a fall was observed in the 4⁺ year-class. The degree of hydration again rose slightly in the fishes of 5⁺ year-class. The water content of the muscle maintained a reciprocal relationship with the muscle fat content (Fig. 23). This relationship was expressed by the following equation:

$$\text{Log } y = 5.5216 - 1.0757 \log x$$

where, y and x were the quantities of fat and water (mg/100 g) respectively. The correlation coefficient (r) for this relation

ship was found to be -0.662±0.102, significant at 0.0 1 level of probability.

Ash content of the muscle did not appear to follow any regular pattern of variation during the growth of the fish. Ash content was found to vary from 1.082 to 1.553% in the fishes of 0* - 5* year-classes. From a value of 1.340 in the specimen of 0* year-class the ash content declined to an all time low in the fishes of 1* year-class and thereafter the ash content again seemed to rise in the successive year-classes from 2* - 4*, but a decline was again noted in subsequent year-class.

DISCUSSION

It was evident from the low muscle protein contents of the fishes of 0* and 1* year-classes that the high protein demands of the rapidly growing body were met partly at the expense of muscle proteins. Indeed, the growth of this species has been reported to be rapid during the first two years of life, and thereafter it declined gradually (Bhatt, 1970). Further decline in the protein content in the 2* - 3* year-classes seemed to be due to the attainment of sexual maturity. A considerable part of the food consumed by the fish appeared to be used up for the ripening of gonads. Reduction of the muscle protein during early life has also been reported in some freshwater fish species by Khawaja and Jafri (1967, 1968). This

reduction was attributed to the utilization of protein towards active growth and maturation of the gonads. The increase in the percentage of muscle protein observed in *L. striatus* belonging to 4+ year-class may be attributed partly to the recovery from a period of active growth and partly to the acquisition of highly proteinaceous diet, comprising of macrocrustaceans, insects, fry, fingerlings and small fishes.

Though the nature of the diet seemed to influence, in part, the protein content of the muscle, the striking similarities in the protein cycle, as reported by Khawaja and Jafri (1967, 1968) in various species of freshwater fishes whose food preferences were very different, suggested that the protein content in fish was largely influenced by growth and maturation and not so much by the quality and quantity of food consumed. McCay and Tunison (1937), however, maintained that the consumption of the protein rich food was responsible for elevating the protein level of the muscle.

Muscle protein content did not seem to alter markedly in fishes of the 5+ year-class, presumably due to the old age when the growth rate has slowed down considerably and, consequently, the ratio of the maintenance and growth food changed in the direction of an increase in the relative amount of maintenance food.

The pattern of changes in the muscle fat content of *L. striatus* during growth seemed to be very similar to that of

protein except that the changes in fat were more pronounced. The decline in the muscle fat in successive year-classes from 0* - 1* appeared to be due to its utilization during rapid growth, while further decline in the fishes of 2* - 3* year-classes seemed to be the consequence of maturation. Since fat, because of its high calorific value (9.3 calories per g), has been known to be a principal source of energy for the whole body, its progressive reduction during growth may be the net result of its utilization for various life processes such as growth, activity rhythm, maturation, etc. (Ahsan and Jafri, 1967a. Furthermore, experiments on the effect of different types of foods on the growth of the carps suggested that the foods with greater calorific value usually result in the most rapid growth (Nikolsky, 1963).

Like protein, the rise in the muscle fat content in fishes of the 4* year-class has been attributed to the recovery from a period of muscle fat deprivation towards active growth of the body. The fish, then, continued to become leaner as it advanced further in age. The dynamics of the coefficient of muscle fat content seemed to be growth specific.

The variations in the degree of hydration of the muscle seemed to be the function of fat dynamics which maintained an inverse correlation with the water content.

Ash content of the muscle did not seem to follow any regular pattern with the growth of the fish. Herrera and Munoz (1957), who observed a seasonal rise in the ash of Sardina pilchardus, suggested that it correspond with the period of maximum growth, which it preceded. Khawaja and Jafri (1967a) recorded a fall in the ash content of the muscles of O. punctatus from 0* - 3* year-classes and thereafter the ash content was reported to increase. These changes were attributed to variation in the mineral contents such as phosphorus, calcium and iron, etc. Thus, as Love (1970) pointed out the only appropriate conclusion that can be derived is that as the proper functioning of the muscle depends upon a correct balance of water, protein and inorganic constituents, it is logical to expect a readjustment of the inorganic substances when the proportions of water and protein alter during the successive periods of growth of the fish.

SUMMARY

Changes in the proximate chemical composition of the muscle were examined during the growth of a freshwater murrel, Ophicephalus striatus Bloch. A decline was noted in the muscle protein and fat contents in the fishes of successive year-classes from 0* - 3*. This decline was attributed to the diversion of these reserves towards active growth and maturation. In the fishes of 4* year-class, however, the values again rose,

indicating a recovery from the period of active growth. Slowing down of growth beyond this phase was characterized by less pronounced changes in the proximate chemical composition of the fish. The water content of the muscle was found to maintain a reciprocal relationship with the fat and this relationship was expressed by a logarithmic equation. The amount of ash did not follow any regular pattern of variations during the successive periods of growth.

CHAPTER IV

RNA AND PROTEIN SYNTHESIS DURING THE GROWTH OF *CHITOPHALUS PUNCTATUS* BLOCH.

INTRODUCTION

Studies on the growth of natural fish populations constitute an important aspect of many applied problems of fisheries management. Since growth of fish is influenced by a combination of physiological and ecological factors, the age can not be taken as the only criterion for its assessment. Fish growth, therefore, can be visualized as a series of growth stanzas which are entered by physiological and ecological size thresholds and within which increase in size or flesh is the basic determinant of both physiological and ecological opportunity for growth (Parker and Larkin, 1959). As this increase in size or flesh is accomplished through the synthesis of body constituents, chiefly protein, useful measures of the growth process would be the measures of protein and the organizer (ribose nucleic acid, RNA) of protein synthesis (Bulow, 1970).

MATERIALS AND METHODS

Methods used for the collection and maintenance of fishes, determination of their age, muscle sampling, quantitative estimations of RNA and protein, and the statistical evaluation of the

Fig. 24. Changes in the RNA and protein concentrations in the flesh of *O. punctatus* during growth (circles represent mean; vertical lines indicate standard error).

data, were the same as described under 'Procedure and Methodology' (pages 9-33).

RESULTS

The values of RNA and protein in Ophiocephalus punctatus, belonging to various age groups, have been presented in Table 8 and Fig.24. As would be evident from the data, the RNA content declined from 119.643 µg/100 mg in the 0+ age-group to 100.195 µg/100 mg in the subsequent year-class when the fish attained maturity, but thereafter, the value continued to increase with the growth of the fish. In the specimens of 5+ age-group the concentration of RNA thus rose to 150.150 µg/100 mg.

The trend of change in the protein concentration in various year-classes of O. punctatus was similar to that of RNA, excepting that the changes in protein were less drastic.

The attainment of old age was characterized by relatively little changes in both RNA and protein contents.

Within the range investigated, the levels of RNA and protein appeared to maintain a direct proportionality. The logarithmic transformation of this relationship was expressed through the following regression equation:

$$\text{Log } x = -2.4613 + 3.6492 \log y$$

where x was the µg RNA/100 mg tissue (wet weight) and, y was the mg protein/100 mg tissue (wet weight).

The correlation coefficient, r, (0.917±0.037) for this relationship was found to be significantly high ($P < 0.001$).

DISCUSSION

The growth of a fish, which is largely a function of increase in the amount of protein and the RNA, which plays a key role in its synthesis, results from the consumption of food, its assimilation and incorporation in the body.

The presence of substantial quantities of RNA in tissues synthesizing large amounts of protein, for either the growth or multiplication, has been reported by Brachet (1955). In fishes, as the growth in the bulk of the skeletal muscles is related to an increase in the size rather than in the number of cells (Love, 1958c), the rise in the levels of RNA and protein, therefore seems to indicate the growth and not the multiplication within the tissue.

The close similarity observed in the pattern of variations of RNA and protein in *L. punctatus* of various year-classes, associated with different growth characteristics, points towards the existence of close quantitative relationship between the two.

The period of most rapid growth, which occurred before the onset of sexual maturity in young fishes of 0+ age-group, was associated with higher levels of RNA and synthesis of considerable quantity of protein than in fishes of the succeeding year-class. The utilization, in the pre-maturity stage, of the major portion of food-intake towards the synthesis of protein in preference to other organic constituents has been known to occur in many fish species (Nikolsky, 1963). The faster growth in young fish has been regarded as an adaptive property which, besides offering protection against predators by virtue of the increased size (Nikolsky, 1963), has also been reported to enable the fish to withstand the extremes of temperature fluctuations of the environment through slowing down the rate at which the temperature of the body tissues could change in accordance with the changes in the water temperature (Hoar, 1955).

Decline in the values of both RNA and protein in the muscles of the fishes of 1+ age-group was coincident with the onset of maturity, whence considerable part of the food consumed by the fish was diverted towards gonad-building. Evidences of the loss of RNA from the flesh of fish during maturation have also been presented by Crossman and Tomlinson (1959) and Krishnamma Rao (1965). The depletion of muscle protein during maturation has, however, been well documented (Delbrido et al., 1960; Khawaja and Jafri, 1967a,b, 1968).

The increase in the RNA and protein concentrations observed in the muscle of fishes after 0+ age-group was evidently the consequence of the acquisition of highly proteinaceous diet, comprising mainly of forage fishes. This changeover from a planktivorous to a piscivorous habit of this species was reported by Tandon (1962). From the fact that a mere change from a plankton-feeding to a fish-feeding habit has the potential to enhance the growth rate of the fish (Martin, 1966), it could be concluded that the protein-rich diet increases the RNA and protein content of the body.

Brachet (1955) has concluded that the RNA content could be determined mainly by the protein content of the diet, and protein synthesis in the body was linked with the dynamics of the change in the levels of RNA. Loss of RNA and protein from the body with consequent slowing down or even complete cessation of growth during protein-free diet or fasting as reported by Leslie (1955) give further support to the present findings on L. mugilaris.

Relatively little change in the concentrations of RNA and protein observed in higher age-group (5+) accompanied considerable fall in the growth rate of the fish at this stage of life as has been reported by Qasim and Bhatt (1966). This was invariably the outcome of a change in the ratio of maintenance to the growth-food, i.e. the direction of an increase in the relative amount of maintenance food, which has been known to

characterised the period of old age in fish (Nikolsky, 1963).

SUMMARY

Changes were studied in the concentrations of RNA and protein during the growth of Channacephalus punctatus. The pattern of changes in the RNA and protein contents in different age-groups of the fish were almost similar, though the changes in the latter were less marked. The decline in the levels of the two constituents in the specimens of 1+ age-group seemed a consequence of the attainment of maturity, while increase in their concentrations in the succeeding year-classes was the result of the acquisition of a highly proteinaceous diet. The increase in the logarithm of protein as a direct function of the increase in the logarithm of RNA (r = 0.917) revealed the importance of this nucleic acid in the synthesis of protein in the growing fish.

CHAPTER VII

RNA AND PROTEIN CONTENT IN RELATION TO BIOLOGICAL 'CONDITION' OF *CHICHLASOMA PUNCTATUS* BLOCH.

INTRODUCTION

The elucidation of the biological role of ribose nucleic acid (RNA) as an organiser of protein synthesis increases upon the need to study if this chemically demonstrable relationship can be related to the 'condition' of the fish, as determined from the relationship between its length and weight. The use of the RNA measurement in fish to assess the growth rate, which is largely a function of protein synthesis, pointed in this direction (Hulow, 1970). Attempts to apply the quantitative estimates of nucleic acids for the prediction of growth rate and measurement of biomass production of marine phytoplankton and zooplankton have indeed yielded fruitful results (Hutcliff, 1965; Holm Hansen *et al.*, 1968).

This chapter gives an account of the changes in the levels of RNA and protein as related to the 'condition' in *Cichlasoma punctatus* Bloch. In these investigations the 'fillet condition factor' rather than the condition factor of the fish as a whole, was used, since fillets provide the major contribution to the very bulk of the body, and it is on the tissue sample from the fillet that the chemical estimations have been based. Further,

the fillet condition factor is more constant as it is least affected by factors like the diurnal rhythm in feeding activity.

MATERIAL AND METHODS

Methods employed for the collection and maintenance of fishes, determination of their fillet condition factor, sampling of muscle tissue, quantitative estimations of RNA and protein, together with the statistical interpretation of the data have been described under 'Procedure and Methodology' (pages 9-33).

RESULTS

The concentrations of RNA and protein and the values of fillet condition factor of *L. punctatus* of various body lengths have been given in Table 5.

The concentration of RNA in the flesh of *L. punctatus* was found to be a linear function of its fillet condition factor (C). The regression analysis of the logarithmically transformed data gave the equation:

$$\log C = -1.6933 + 1.1392 \log \text{RNA } (\mu g/100 \text{ mg}).$$

The correlation coefficient (r) for this relationship was found to be 0.951 ± 0.002, significant at 0.001 (probability).

Like RNA, the increase in the protein content of the flesh was directly related to the increase in the fillet condition factor. The relationship could be expressed by the following logarithmic equation:

$$\log C = -1.7705 + 2.0403 \log \text{protein } (\text{mg}/100 \text{ mg})$$

The above relationship gave a significantly high ($P < 0.001$) correlation coefficient (0.978 ± 0.001).

Throughout the range of investigation, the increase in the logarithm of RNA was accompanied by an increase in the logarithm of protein. The regression equation establishing this relationship was found to be:

Log RNA (μg/100 mg) = 0.1229 + 1.6398 log protein (mg/100 m

The correlation coefficient (0.940 ± 0.002) was statistically significant at 0.001 level.

DISCUSSION

In fishery biology, the term 'condition' signifies the various biological features of fish, such as robustness, suitability of environment and gonad development. The 'condition' in most fishes varies according to the season and locality (Love, 1962). Variations in the 'condition' of the fish are caused mainly by the non-seasonal changes in the physiology of the fish and the fitness of environment, specially with relevance to its nutritional richness.

In *Q. punctatus* the RNA and protein concentrations in the flesh were observed to be a linear function of the fillet condition factor. [illegible] the positive correlation between the protein content and condition factor are those of Ironside

and Love (1958) and McComish et al. (1974). Since the present experiment was completed within a fortnight, the possible influence of the seasonal variations was minimised, and the changes in the fillet condition factor, together with the accompanying changes in the RNA and protein, seemed particularly the direct consequence of the nutritional status of the fish. Dulow (1970) has reported an increase in the RNA concentration with increase in the feeding level of a fish. Increase in the RNA and protein levels with increase in the dietary intake of the fish was also evident in other reports (Brachet, 1955; Leslie, 1955).

The coincidence of high RNA concentrations with high concentrations of protein indicated the role of this nucleic acid in protein synthesis. Strong quantitative relationships between RNA and protein, as established through the regression models in the present study, were also documented by Brachet (1955) in the silk gland of silk worms, various organs of vertebrates, and more impressively in the growing cultures of micro-organisms. Further experimental studies carried out by Venugopalan (1967) on the effect of the administration of growth hormone on RNA level, and by Leslie (1955) on the RNA concentration in regenerating tissues after partial hepatectomy, suggested that the increase in the RNA content remarkably coincided with an increase in the growth rate. Studies reported by Brachet (1955) on the simultaneous effect of various chemical substances on the turnover rates of both the RNA and protein furnished furth

evidence in favour of the very close relationship existing between the RNA and protein synthesis.

From the results of the present study and the diverse evidences cited to support these, it became evident that the growth of the fish, which is accomplished mainly through the synthesis of protein, is closely linked with the synthesis of RNA, thereby demonstrating the use of this nucleic acid as a biochemical indicator of the recent growth rate of fish.

SUMMARY

Ophicephalus punctatus Bloch. was analysed to study the concentrations of RNA and protein in relation to its living condition. The levels of both RNA and protein were related to the fillet condition factor. The synthesis of protein was invariably associated with the dynamics of the change in the levels of RNA.

CHAPTER VIII

CHANGES IN THE PRINCIPAL CHEMICAL COMPOSITION, NITROGEN BALANCE AND ENERGY LOSSES IN THE CAT-FISH, *HETEROPNEUSTES FOSSILIS* (BLOCH) IN RELATION TO STARVATION

INTRODUCTION

A unique feature of fishes is their ability to withstand periods of prolonged starvation through physiological and biochemical changes in their body. Besides that their basal energy consumption is known to be generally lower as compared to terrestrial animals, their metabolic demands decline further during starvation (Love, 1970). Added to this is their adaptation to mobilise their body constituents as fuel for survival through the activity of autolytic enzymes which remain in readiness for such periods of total abstention from food (Siebert *et al.*, 196

The present study was undertaken to investigate the changes in the principal body composition, energy content, nitrogen balance and the inter-relationship, if any, between some chemical constituents during starvation of a freshwater catfish, *Heteropneustes fossilis* (Bloch). A record was also made of the dynamics of the 'fillet condition factor' inasmuch as it can reflect the declining levels of body constituents and from physiological view point, can be used as an index of the living state of the fish during starvation.

MATERIALS AND METHODS

Methods used for the capture, maintenance, tagging of live specimens of Heteropneustes fossilis, removal and measurements of fillet for the determination of fillet condition factor, sampling of tissue, chemical analyses and statistical expression of the data, were the same as outlined under 'Procedure and Methodology' (pages 9-33).

RESULTS

I. CHANGES IN WEIGHT

A steady decline in the weight of the fish occurred with starvation (Table 10). For a fish of the initial weight of 50 g, the decline in the weight for each 10-day's interval up to 50 days of starvation was of the order of 5.055, 6.599, 8.125 9.604 and 10.487 g, respectively (Table 11). On an average, the loss in the body weight was 209.740 mg/day. The regression analysis of the logarithmically transformed data of weight corresponding to the period of starvation gave the equation:

$$\text{Log } W = 1.7207 - 0.0701 \log D$$

where, W was the weight (g), and D was the number of day of starvation.

The correlation coefficient, r (-0.954) for 14 degrees of freedom was found to be significant at 0.001 level of probability.

Fig. 25. Changes in the principal chemical constituents (Protein, ; Fat, ; Water, ; Ash,) of the flesh during starvation.

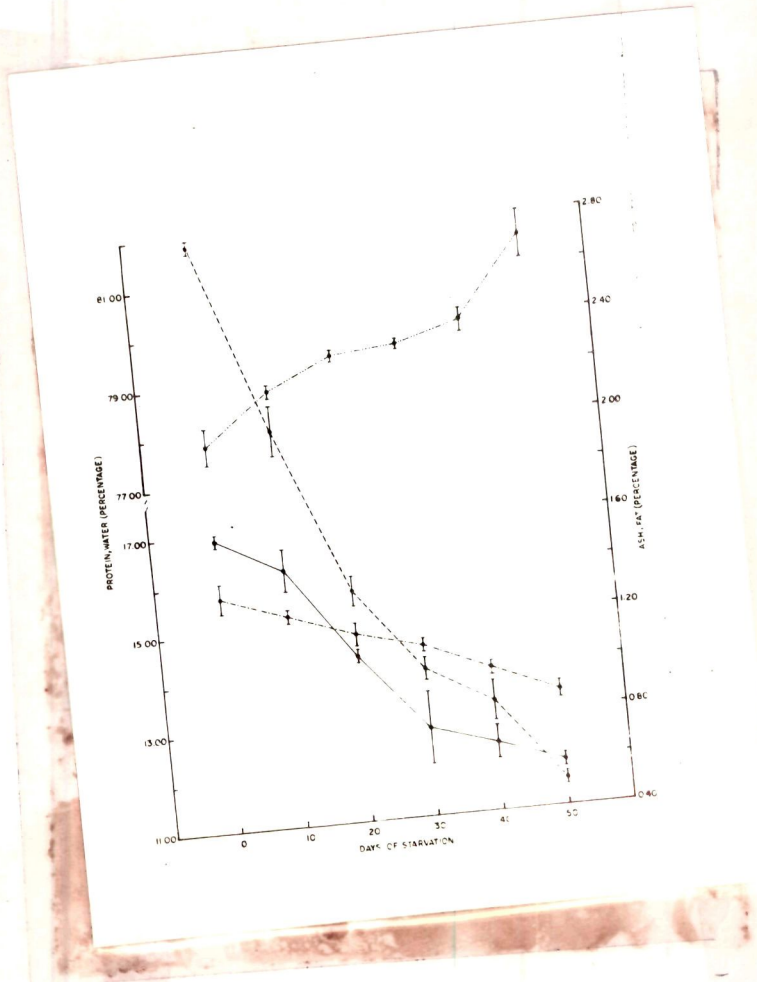
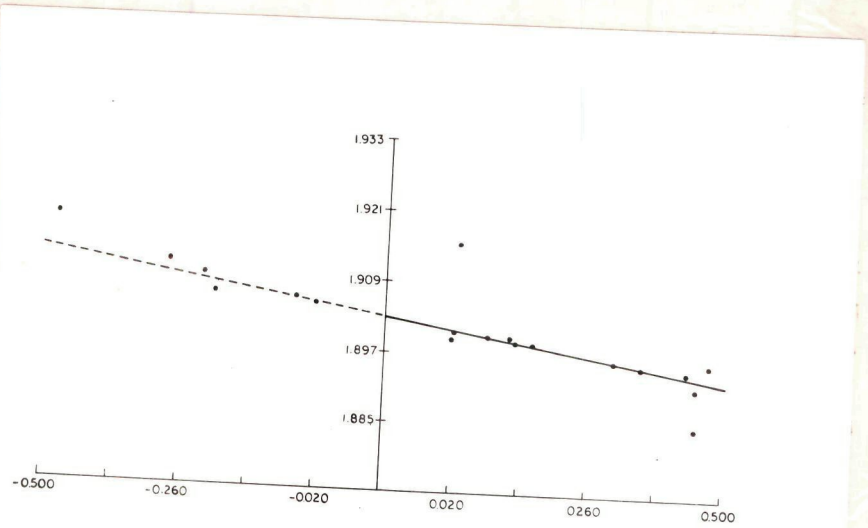

81.00
79.00
77.00
17.00
15.00
13.00
11.00
PROTEIN, WATER (PERCENTAGE)
2.80
2.40
2.00
1.60
1.20
0.80
0.40
ASH, FAT (PERCENTAGE)
0
10
20
30
40
50
DAYS OF STARVATION

Fig. 26. Fat/water relationship during starvation.

Abscissa : log fat, percentage.

Ordinate : log water, percentage

(Intercept = 9.8200, slope = -5.1625).


1.933
1.921
1.909
1.897
1.885
-0.500
-0.260
-0020
0.020
0260
0.500

In the cat-fish investigated, the fillet which constituted the major portion of the total body weight seemed to be highly affected by the stress of starvation. This was evident from the changes in the fillet weight as indicated through the fillet condition factor which were of the declining order during the progress of starvation (Table 10).

II. CHANGES IN THE PRINCIPAL CHEMICAL CONSTITUENTS

The cumulative weight loss of the fish during starvation appeared to be the result of fall in the absolute concentration of each of the principal body constituents (Table 11). However, when the values of these constituents were expressed through their percentages, a decline in the fat, protein and ash while a relative increase in the water was observed (Fig. 25 , Table 12).

1. <u>Fat content</u>. While a sharp decline in the fat content was found to occur from the very start of starvation, the rate of its fall seemed to have declined after the onset of 40 days of starvation. By the end of 50 days, a significant ($P < 0.001$) fall of 81.300% in the fat level was observed.

2. <u>Water content</u>. The percentage of water, which increased during starvation, maintained an inverse relationship with the percentage of fat (Fig. 26). This relationship was expressed through the equation:

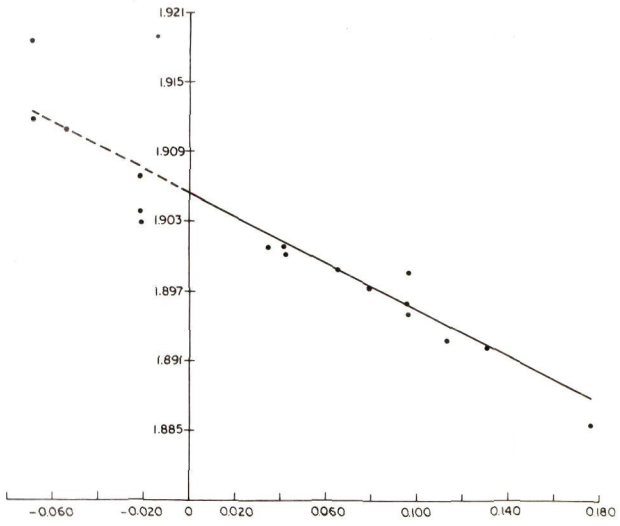
$$\text{Log } F = 9.8200 - 5.1625 \log M$$

Fig. 27. Relationship between ash and water during starvation.

Abscissa : log ash, percentage.

Ordinate : log water, percentage

(Intercept = 2.5900, slope = -1.3605).


1.921
1.915
1.909
1.903
1.897
1.891
1.885
-0.060
-0.020
0
0.020
0.060
0.100
0.140
0.180

where, F was the percentage of fat and M was the percentage of water (moisture).

The correlation coefficient, r for 17 degrees of freedom was found to be -0.980, significant at 0.001 level of probabilit The sum of fat plus water varied from 80.580% to 80.859% during the first 40 days of starvation. This value rose to 82.021% when the period of starvation was extended to 50 days.

3. Protein content. Changes in protein content, during the first 40-days of starvation remained relatively less pronounced (Fig. 25). The mobilisation of protein, however, appeared to have been triggered by 20 days of starvation. When the fish was starved for 50 days a fall of 30.590% was noted. When the value of protein obtained before the onset and after the completion of starvation were compared and the difference tested statistically by student's t-test, the result was found to be significant at $P < 0.001$.

4. Ash content. The percentage of ash declined with the period of starvation, till the end of 50 days when a fall of about 34.5% was observed. This decline in the ash level, as assessed statistically was found to be significant at $P < 0.02$.

A reciprocal relationship also seemed to exist between the ash and water contents (Fig. 27). The regression analysis of this relationship for 17 degrees of freedom gave the logarithmic equation:

$$\text{Log } A = 2.5900 - 1.3605 \log M$$

where, A was the ash percentage and W was the percentage of water.

The value of correlation coefficient, r (-0.970) for the above relationship was found to be significantly high ($P < 0.01$).

III. NITROGEN EQUILIBRIUM

A progressive decline in the nitrogen concentration was associated with the period of starvation. As the replenishment of nitrogen from the exogenous sources was cut off, the amount of nitrogen excreted became an index of the quantity of the tissue protein catabolized. During the present study, the amount of nitrogen excreted at each 10-days interval, up to a total of 50 days of starvation, were 82.200, 203.600, 371.600, 426.000 and 485.200 mg, corresponding to a weight loss of 5.855, 6.599, 8.123, 9.604 and 10.487 g. This gave an average value of 9.704 mg nitrogen excreted per day and a weight loss of 209.740 mg in one day. Thus, 9.704 mg of endogenous nitrogen excreted indicated a fall of 209.740 mg of indispensable body tissue. The logarithmic transformation of the amount of nitrogen excreted and the loss in body weight gave the equation:

$$\log N = -0.0917 + 2.7926 \log w$$

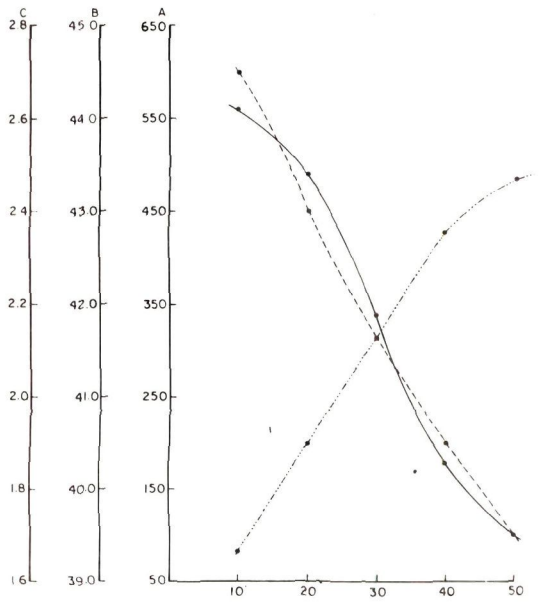
where, N was the amount of nitrogen excreted (mg) and w was the weight loss (g).

The correlation coefficient, r for this relationship was significant at 0.001 level of probability.

Fig. 28. Negative nitrogen balance ( )
in relation to changes in weight ( )
and fillet condition factor ( ).

Abscissa : Days of starvation,

Ordinate : A, endogenous nitrogen excreted (mg),
B, decline of weight (g),
C, fillet condition factor.


C
2.8
2.6
2.4
2.2
2.0
1.8
1.6
B
45.0
44.0
43.0
42.0
41.0
40.0
39.0
A
650
550
450
350
250
150
50
10
20
30
40
50

The excretion of endogenous nitrogen and the decline in the weight of the fish in relation to fillet condition factor have been shown in fig. 28 .

IV. ENERGY LOSSES

The energy losses corresponding to the mobilization of protein and fat were estimated during the present study in fish of 50 g of initial body weight. A decline in the energy content with starvation was evident (Table 12). Regression analysis of this energy loss gave the equation :

$$\text{Log } E = 1.943^{*} - 0.3640 \log D$$

where, E was the energy (calories), and D was the number of days of starvation.

The value of correlation coefficient, r (-0.965) was highly significant ($P < 0.001$).

DISCUSSION

Although the decline in the total body weight was one of the basic manifestations of starvation, this was due mainly to the loss in the weight of the fillet which formed the main bulk of the fish body. Under prolonged starvation the changes in the fillet weight, as expressed through 'fillet condition factor', indicated more accurately the processes of depletion going on in the body than the changes in the total body weight (Wilkins, 1967).

In general, the decline in the fillet condition factor during starvation was characterised by a cumulative loss in the weight indicative of fall in the absolute levels of the principal chemical constituents of the body (Table 11). However when relative values of these constituents were expressed as percentage of wet weight the values of protein, fat and ash seemed to decline as against a reciprocal increase in the level of water (Fig. 25).

A sharp depletion of fat from the very start of the starvation indicated the preference with which this high energy constituent was drawn upon to meet the metabolic requirements of the body. The declining values of fat were accompanied by increase in the degree of hydration of the tissue. Such an inverse relationship between fat and water during starvation has also been reported by Love (1958), Wilkins (1967) and Hilal (1972). The assumption that by increase in the proportion of one of these constituents with a simultaneous decrease in the other, the sum of the two remained relatively constant (Black and Schwartz, 1950; Idler and Bitners, 1959; Coppini, 1967; Love, 1970) was found to be true during the period when much of the calorific requirements were maintained at the expense of endogenous fat. In the succeeding stages when the fat percentage was reduced to as low as 0.5% the constancy in the sum of fat plus water was altered. This could be due to the mobilisation of protein and hence to the protein-water effect.

But as this difference in the sum of fat and water was only 1.126%, the fat - water relationship did not deviate to any great extent from the straightline obtained by the logarithmically transformed data.

Inasmuch as the fat percentage in the fish studied was only 2.707% on fresh weight basis, the protein content also seemed to be mobilized. Changes in the protein content, though little, started from the very beginning of starvation. A sharp decline in protein content, during the latter stages of starvation indicated that the declining fat reserves could not sufficiently provide energy for the metabolic activity, and perhaps this might have provided the stimulus to enhence the mobilisation of muscle protein before the tissue were completely depleted of their fat content. Reduction in the endogenous protein before complete utilization of fat resources has also been reported by Greene (1926) and Templeman and Andrews (1956). This mobilisation of protein during starvation thus appeared to be a requisite for sustaining the life of the fish, when the concentration of this constituent was considerably higher than that of the indispensable fat.

Since the exogenous sources of the energy food stuffs were cut off, the depletion of both protein and fat during starvation indicated the proteolysis and lipolysis of the essential endogenous protein and fat, with a resultant disruptio of cellular metabolism (Wilkins, 1967).

The breakdown of protein was seen to result in the fall of nitrogen level in the body. This decline not only represented an alteration in the proportion or percentage of total nitrogen but an actual decrease in the amount of total nitrogen. Concordant to this observation was the finding of Wilkins (1967) on the herring, Clupea harengus, which was even more fatty than the cat-fish, Heteropneustes fossilis, studied here. Since nitrogen has been regarded as the most characteristic and constant element of proteins and of the products of protein metabolism (West and Todd, 1961), it was possible to determine the overall metabolism or protein by determining the so-called nitrogen balance. This nitrogen balance could be maintained through the intake of proteinaceous food (Stanley, 1974). During food deprivation, however, the nitrogen loss could not be replenished and the conditions of negative nitrogen equilibrium were bound to prevail.

In fish of 50 g initial weight the rate of endogenous nitrogen excretion during 50-day starvation was of the order of 9.704 mg N/day. As this excretion of nitrogen was exclusively at the expense of structural proteins, which in the species investigated contributed more to the total weight than the fat and ash combined, the quantity of the nitrogen lost could be used as an index of the tissue loss.

The energy losses, as they run parallel with the period of starvation, could be equated to the total energy equivalents

calculated from the amounts of indispensable tissue protein and fat utilized by the fish. The loss in energy value, therefore, was largely a function of the declining fillet condition factor of the fish.

It was clear from the present investigation that the percentage of ash declined with the duration of starvation. This decline in the ash was so consistent with increase in the percentage of water that the dynamics of the change in one of these constituents reflected an inverse sequence of variation in the other. Divergent views have, however, been expressed as to the changes in the ash content during starvation. While some workers (Phillips et al., 1960; Wilkins, 1967; Hilmi, 1972) observed increase in the amount of ash during starvation, others (Tilik, 1932; Koriyl, 1951; Love, 1958; Love et al., 1968) reported decline in the ash content of the body. It was rather striking that ash was reported to increase in cases where whole fish, including the bones and the skin, was analysed, while in cases where bone- and skin-free muscle was examined the ash was seen to decrease with starvation. It was, therefore, evident that the bones and the skin which have been known to be rich in ash (Young and Lorimer, 1960; Love, 1970) increase in relative proportion and become sources of increase in the total (whole) body ash. However, in the fillets cleared of bones and skin, the fall in the percentage of ash was an invariable accompaniment of starvation.

SUMMARY

A prolonged deprivation of the cat-fish, Heteropneustes fossilis, from food results in a state of physiological emergency, during which the structural proteins and fats are hydrolysed to meet the energy requirements of metabolism. The excretion of nitrogenous products, formed at the expense of sarcoplasmic protein, causes a negative nitrogen balance inasmuch as the replenishment from exogenous sources remains withheld. The loss of endogenous chemical constituents and energy value, together with a relative increase in the hydration of the tissues and a fall in the level of inorganic substances, brings about a disorganisation of metabolic activity at the cellular level.

CHAPTER IX

PHOSPHATASES AND 5'-NUCLEOTIDASE IN THE DARK AND WHITE MUSCLES OF TWO SPECIES OF FRESHWATER CAT-FISHES, CLARIAS BATRACHUS (LINN.) AND HETEROPNEUSTES FOSSILIS (BLOCH).

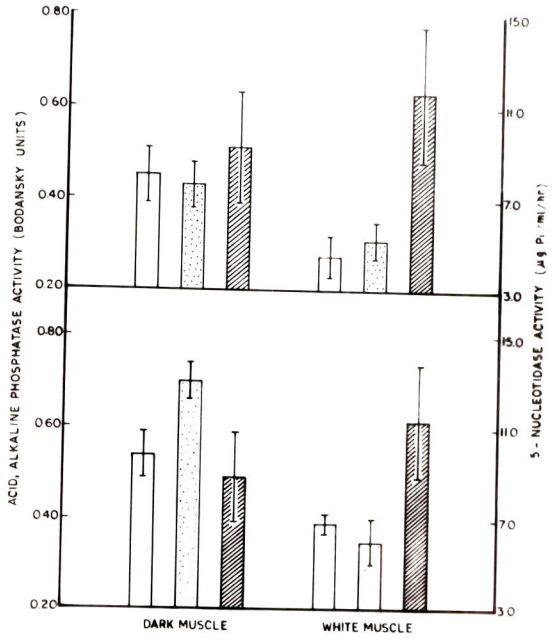
INTRODUCTION

A survey of the literature shows that despite considerable reports on fish enzymes, mainly in relation to processing and spoilage (Tarr, 1955, 1956, 1958; Ono *et al.*, 1957; Siebert, 1958 Connel, 1960; Jones, 1962; Markert and Faulhaber, 1965; Love, 1966; Partmann, 1969; Ramohar, 1970; Wojkowics and Odense, 1972; Hilts *et al.*, 1974; Nowlan and Dyer, 1974), very few investigations have been directed towards a comparative study of different enzymes in the dark and white muscles (George, 1962; Pora *et al.*, 1965). There seems to be almost no information on the activity of alkaline (EC 3.1.3.1.) and acid EC 3.1.3.2) phosphatases and that of 5'-nucleotidase (C 3.1.3.5.) in the two types of fish muscles. The present chapter reports the biochemical localization and the functional significance of these enzymes in the dark and white muscles of two common freshwater cat-fishes, Clarias batrachus (Linn.) and Heteropneustes fossilis (Bloch.).

MATERIALS AND METHODS

Details of the procurement and rearing of live specimens of C. batrachus and H. fossilis, tissue sampling, analytical techniqu

Fig. 29. Activity of acid phosphatase (white bars), alkaline phosphatase (dotted bars) and 5'-nucleotidase (striped bars) in the dark and white muscles of C. mrigr (bottom) and H. fossilis (top). Vertical lines indicate standard error mean.


0.80
0.60
0.40
0.20
0.80
0.60
0.40
0.20
ACID, ALKALINE PHOSPHATASE ACTIVITY (BODANSKY UNITS)
150
110
70
30
150
110
70
30
5 - NUCLEOTIDASE ACTIVITY (µg P. / ML. / HR.)
DARK MUSCLE
WHITE MUSCLE

and methods of calculation, were the same as outlined under 'Procedure and Methodology' (pages 9-33).

RESULTS

The activities of the acid and alkaline phosphatases and 5'-nucleotidase in the dark and white muscles of C. marmur and H. fossilis have been given in Table 13 and shown graphically (Fig. 29).

Interspecific differences - Interspecific variations seemed to occur in the activities of the three enzymes. The levels of acid phosphatase in the dark and white muscles of C. marmur were higher than those in the dark and white muscles of H. fossilis. Like its acid counterpart, the alkaline phosphatase activity in the dark and white muscles of C. marmur were also higher in comparison to the levels of the activity of this enzyme in the two muscles of H. fossilis.

Unlike the phosphatases, the 5'-nucleotidase activity in the dark and white muscles of H. fossilis was found to be greater in contrast to the levels of this enzyme in the dark and white muscles of C. marmur.

Differences between dark and white muscles - As compared to the white, the dark muscle of both the teleostean species, C. marmur and H. fossilis contained contained higher levels of acid phosphatase activity.

The alkaline phosphatase activity too, was found to be greater in the dark muscles of both the species.

The distributional characteristic of the 5'-nucleotidase in the two muscle types of the teleostean species was found to be reciprocal to that of the phosphatases. In white muscle of C. magur and H. fossilis the 5'-nucleotidase activity was higher than that in the dark muscle of the two species of cat-fishes.

DISCUSSION

The presence of acid and alkaline phosphatases in tissues has been known to be of great significance for the various metabolic processes. Attempts to study the metabolism of phosphorylated derivatives of naturally occurring organic compounds have revealed the existence of these enzymes that catalyze their hydrolysis (Heppel, 1961). The role of phosphatases in the metabolism of carbohydrates and phospholipids has also been well documented (Bradfield, 1957). In the present study, the occurrence of higher activities of the acid as also of the alkaline phosphatases in the dark muscle might be due to active hydrolysis of the esters of phosphorylated compounds or to the metabolism of greater quantities of carbohydrates and phospholipids, both of which have been reported to be more concentrated in the dark muscle (Draekkan, 1956; Kataoka et al., 1960; Olley et al., 1962; Buttkus, 1963; Bone, 1966).

The greater activity of the two phosphatases in the dark muscle also suggested that the activity of one of the enzyme was not affected by the activity of the other, in the same tissue, in performing their physiological roles. The variations of intra-cellular pH appeared to allow the two enzymes with different pH optima to act simultaneously to the advantage of the economy of the living matter (Hoog, 1946).

In contrast to the two phosphatases, a higher activity of 5'-nucleotidase in the white muscle may be attributed to the greater mechanical activity of this tissue that required a delicate mechanism for the regulation of the cycle of inorganic phosphorus (Pi) and adenosine monophosphate (AMP), which have been regarded as the products of the action of 5'-nucleotidase on the nucleotide molecule. This should not, however, exclude the possibility of the role of the dark muscle in the mechanical activity of the fish. The turnover of considerable quantities of lactic acid in the dark muscle during exercise (Wittenberger and Macleuc, 1965) pointed to its role in the mechanics of muscular activity. Perhaps, it might be through the transfer of metabolites to the white muscle which is known to be the first to be affected by the muscular stress. A close proximity of the dark to the white muscle in the two teleostean species examined seemed to be significant in this respect. Other studies on the relative concentrations of energy rich phosphate compounds are rather inconclusive. Kutscher and Ackermann (1933) and Nakano (1961) have reported

the presence of higher concentrations of creatine phosphate in the dark muscle, to supply energy for muscular contraction, while Nakano and Tsuchiya (1960), Sakaguchi et al. (1964) and Fraser et al. (1966) found no distinctive distribution of high energy phosphate compounds, including creatine and ATP, in the dark and white muscles of the fish. Hamoir (1955), on the other hand, reported a higher capacity of the dark muscle synthesize the energy rich phosphate compounds.

From the standpoint of fish technology, it was worth noticing that the lower activity of 5'-nucleotidase in the dark muscle as compared to the white, with however little difference, as was evidenced from the present data, could become limiting to the availability of ATP and hence to the formation of inosine mono-phosphate (IMP) which is known to be produced as a result of the action of ATP-deaminase on the ATP, and is responsible for the development of flavour in fish meat.

SUMMARY

The distribution of the acid and alkaline phosphatases and 5'-nucleotidase was studied in the dark and white muscles of two freshwater cat-fishes, namely, Clarias batrachus and Heteropneustes fossilis. The phosphatases were found to be more concentrated in the dark muscle while the 5'-nucleotidase activity was relatively higher in the white muscle. Higher phosphatase activities in the dark muscle appeared to be a

consequence of active hydrolysis of phosphomonoesters or metabolism of greater quantities of carbohydrates and phospholipids. Higher activity of the 5'-nucleotidase in the white muscle, on the other hand, might be the result of a more intricate control mechanism of the dynamics of inorganic phosphate and ATP.

CHAPTER X

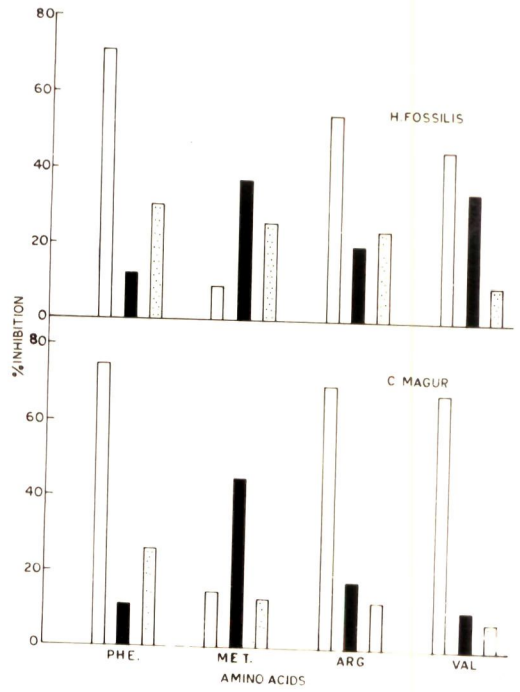
AMINO ACID INHIBITION OF THE ALKALINE PHOSPHATASES OF THE DARK, WHITE MUSCLES AND LIVER OF *CLARIAS BATRACHUS* (LINN.) AND *HETEROPNEUSTES FOSSILIS* (BLOCH).

INTRODUCTION

The alkaline phosphatases (Orthophosphoric monoester phosphohydrolases, EC 3.1.3.1.) are known to be widely distributed in animal tissues. Besides their vital role in the metabolism of carbohydrates, nucleotides and phospholipids (Kummer and Somers, 1955), they are reported to be involved in the liberation of newly synthesized protein from the nucleoprotein complex (Bradfield, 1951).

A number of studies are directed towards the inhibition of alkaline phosphatases by chemical substances (Morton, 1955; Thomas and Aldridge, 1966; Srikantaiah *et al.*, 1967; Ghosh and Fishman, 1968a; Ghosh and Fishman, 1968b; Kalani and Sharmarao, 1969; Reddi, 1969). Among the various inhibitors used, prominent role was attributed to amino acids. The reports on the stereospecific inhibition of the alkaline phosphatase activity by L-phenylalanine (Fishman *et al.*, 1962, 1963), and the species, organ and subcellular specificity of these enzymes as also determined by amino acid inhibition (Lustig and Kellen, 1971), have given a dynamic turn to the study of isoenzymes of alkaline

Fig. 30. Percentage inhibition by various amino acids of alkaline phosphatase activity of the white muscle (white bars), dark muscle (black bars), and liver (dotted bars).


80
60
40
20
0
H.FOSSILIS
% INHIBITION
80
60
40
20
0
C.MAGUR
PHE.
MET.
ARG
VAL
AMINO ACIDS

phosphatases and enabled the identification of their tissue source *in vitro*.

The present study establishes the pattern of amino acid inhibition of alkaline phosphatase activity in the dark and white muscles and the liver of two common species of freshwater catfishes, namely, *Clarias batrachus* (Linn.) and *Heteropneustes fossilis* (Bloch.).

MATERIALS AND METHODS

Methods followed for obtaining live specimens of *C. batrachus* and *H. fossilis*, their maintenance in laboratory aquaria, and sampling of tissues, together with biochemical assays and statistical expression of the data have been described under 'Procedure and Methodology' (pages 9-33).

RESULTS

The normal values of the alkaline phosphatase activity in the dark, white muscles and the liver of *Clarias batrachus* and *Heteropneustes fossilis* have been given in Table 14, while the data on the activity of this enzyme in the tissue samples treated with L-phenylalanine, L-methionine, L-arginine and L-valine have been presented in Table 15. The percentage inhibition of the enzyme activity by various amino acids was plotted in Fig. 30.

DISCUSSION

Of the three tissues of *C. mrigal* and *H. fossilis* investigated, the highest alkaline phosphatase activity was found to occur in the dark muscle (Table 14). Higher concentration of phosphatases in the dark muscle in comparison to the white has also been reported by Ogata and Mori (1963) for vertebrates, in general.

The alkaline phosphatases from the dark, white muscle and liver seemed to have their own patterns of amino acid inhibition. In both the fish species, the enzyme activity of the white muscle was found to be greatly inhibited by L-phenylalanine, L-arginine and L-valine, while L-methionine had its maximum effect on the enzyme activity of the dark muscle. The liver alkaline phosphatase, in each case, was either the least effected or had a moderate response (Table 15).

With respect to each amino acid, the order of inhibition of the alkaline phosphatase activity of the dark, white muscles and liver was similar in the two species of fishes, with L-phenylalanine having the maximum effect on the white muscle and minimum on the dark muscle, while the liver showing a moderate response. L-methionine was observed to have its peak inhibitory effect on the alkaline phosphatase of the dark muscle and least on that of the white muscle. The order of inhibition of enzyme activity of the three tissues in the two

cat-fishes, by L-arginine was: white muscle ____dark muscle ___ liver. The pattern of inhibition by L-valine was of the same order. The extent of enzyme inhibition was, however, found to be species specific. In both the fish species the selective target of the enzyme inhibition by L-arginine and L-valine was the white muscle. In C. marmur the effect of L-valine was more pronounced than that of L-arginine while in H. fossilis the sequence of inhibition was just the reverse. This tendency of differential inhibition by different amino acids was also found to be valid for each of the other tissues examined (Table 15). With most of the amino acids, the percentage of inhibition of the dark muscle alkaline phosphatase was observed to be closer to that of the liver than to that of the white muscle (Fig. 30). The present observation provides further support to the biochemical and physiological affinities reported between the dark muscle and the liver (Braekkan, 1956; Mori et al., 1956; Tsuchiya and Kunii, 1960; Bareto, 1961; Wittenberger and Cros, 1961; Zaum, 1963a), adding to the validity of the so-called 'liver concept' of the dark muscle in fish.

SUMMARY

The specificity of amino acid inhibition of the alkaline phosphatase activity of the dark, white muscles and the liver was studied in C. marmur and H. fossilis. The pattern of amino acid inhibition of the enzyme activity was tissue specific, though the tendency of differential inhibition of the enzyme

activity by different amino acids was species-specific. The trend of the inhibition of alkaline phosphatase activity of a particular tissue of a species by L-phenylalanine, L-methionine, L-arginine and L-valine also seemed specific for that very tissue of that very species.

CHAPTER XI

EFFECT OF MUSCULAR ACTIVITY ON THE CARBOHYDRATE METABOLISM IN *CLARIAS BATRACHUS* (LINN.).

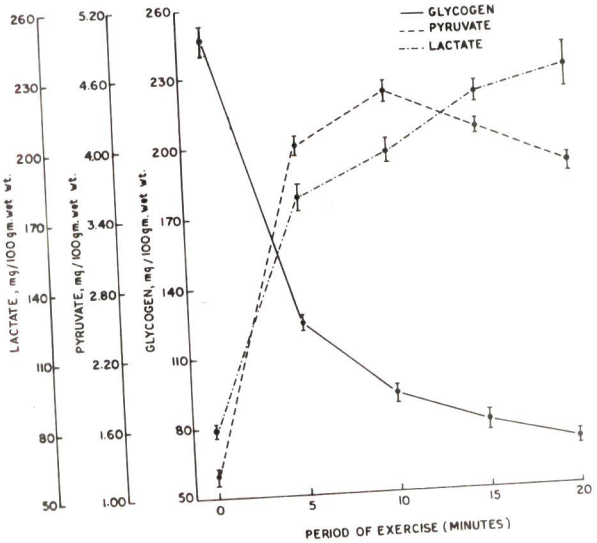
INTRODUCTION

Although the changes in some chemical constituents of fish tissues in relation to muscular activity have been reported by many investigators in the past (Nakatani, 1957; Black *et al.*, 1960, 1962, 1966; Dean and Goodnight, 1964; Wendt, 1965; Stevens and Black, 1966; Bennish, 1968) fewer attempts seem to have been made to furnish a comparative account of the dynamics of metabolic activity in the dark and white muscles (Wittenberger and Diacius, 1965; Bone, 1966; Fraser *et al.*, 1966). The present study was undertaken to investigate the pattern of immediate changes in the carbohydrate metabolism in the dark, white muscles and the liver of *Clarias batrachus* (Linn.). The alterations in blood levels of pyruvate and lactate have also been estimated with a view to elucidate the level of 'excess lactate' and its implications in fish physiology.

MATERIALS AND METHODS

Methods followed for capturing, rearing, exercising the live specimens of *Clarias batrachus*, sampling of tissues and blood,

Fig. 31. Changes in the glycogen, pyruvate and lactate levels in the white muscle during exercise (circles represent mean; vertical lines indicate standard error).


GLYCOGEN
PYRUVATE
LACTATE
LACTATE, mg/100 gm. wet wt.
PYRUVATE, mg/100 gm. wet wt.
GLYCOGEN, mg/100 gm. wet wt.
260
230
200
170
140
110
80
50
5.20
4.60
4.00
3.40
2.80
2.20
1.60
1.00
0
5
10
15
20
PERIOD OF EXERCISE (MINUTES)

chemical analyses, and quantitative interpretation of the data have been presented under 'Procedure and Methodology' (pages 9-33).

RESULTS

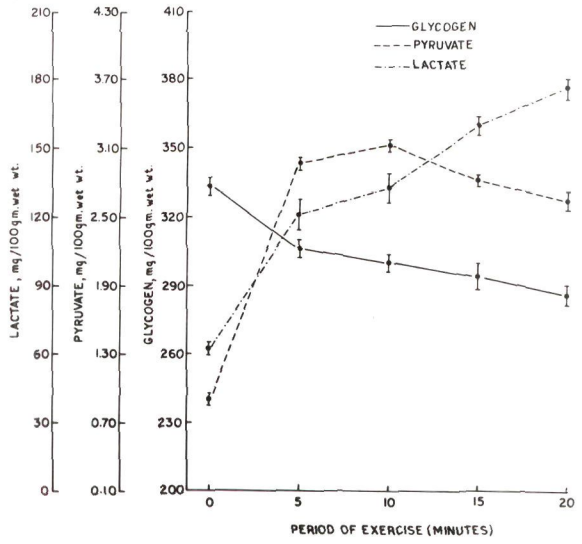
I. GLYCOGEN, PYRUVATE AND LACTATE CONCENTRATIONS OF THE WHITE MUSCLE.

The resting value of white muscle glycogen (246.500 mg/100 g) was subject to drastic changes in the course of muscular activity. The breakdown of glycogen observed with the initiation of muscular activity continued with the period of exercise (Table 16, Fig. 31) to the extent that by the end of 20 minutes it had fallen to 71.400 mg/100 g, a decline of 71.264% from the pre-exercise level.

The pyruvate content of the white muscle, which was 1.141 mg/100 g in the resting condition seemed to rise with the duration of exercise, reaching the peak level (4.453 mg/100 g) after 10 minutes and declining gradually thereafter, though it did not reach the unexercised level (Table 16, Fig. 31).

From a resting value of 70.660 mg/100 g, the lactate level of the white muscle appeared to rise steadily with the period of exercise. A sharp increase was, however, evident after 10 minutes, just when the pyruvate level started declining from an all time high (Table 16, Fig. 31).

Fig. 32. Changes in the glycogen, pyruvate and lactate levels in the dark muscle during exercise (circles represent mean: vertical lines indicate standard error).


GLYCOGEN
PYRUVATE
LACTATE
LACTATE, mg/100gm. wet wt.
PYRUVATE, mg/100gm. wet wt.
GLYCOGEN, mg/100gm. wet wt.
210
180
150
120
90
60
30
0
4.30
3.70
3.10
2.50
1.90
1.30
0.70
0.10
410
380
350
320
290
260
230
200
0
5
10
15
20
PERIOD OF EXERCISE (MINUTES)

II. GLYCOGEN, PYRUVATE AND LACTATE CONCENTRATIONS OF THE DARK MUSCLE.

In contrast to the white muscle, the glycogen concentration in the dark muscle was found to undergo very little change during the course of muscular activity. From a resting value of 333.483 mg/100 g, the dark muscle glycogen declined by only 11.554% during the 20 minutes of exercise (Table 17, Fig. 32).

The pyruvate concentration in the dark muscle was only 0.908 mg/100 g during the resting stage. The value seemed to elevate in the first 10 minutes of muscular activity and, thereafter, it started subsiding gradually, though it did not attain the pre-exercise level (Table 17, Fig. 32).

In the resting fish, the lactate concentration in the dark muscle (61.605 mg/100 g) was observed to increase gradually with the duration of muscular activity. A steep rise in the value seemed to occur as the period of exercise was extended beyond 10 minutes. By the end of 20 minutes, the lactate level in the dark muscle had risen to 175.660 mg/100 g (Table 17, Fig. 32).

III. PYRUVATE AND LACTATE LEVELS OF THE BLOOD.

In the resting fish the pyruvate level in the blood was 0.208 mg/100 ml. This value continued to rise with the period of exercise till a peak level of 0.433 mg/100 ml was attained,

Fig. 33. Changes in the blood levels of pyruvate and lactate during exercise.

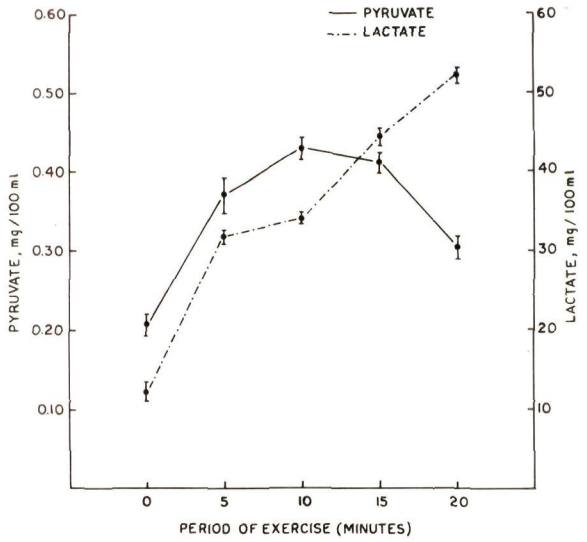
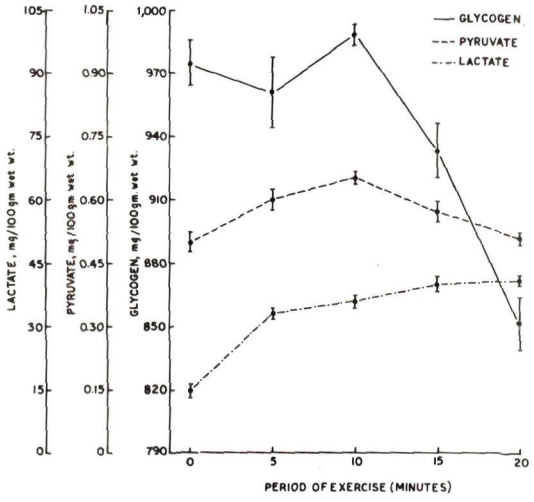

PYRUVATE
LACTATE
0.60
0.50
0.40
0.30
0.20
0.10
PYRUVATE, mg/100 ml
60
50
40
30
20
10
LACTATE, mg/100 ml
0
5
10
15
20
PERIOD OF EXERCISE (MINUTES)

Fig. 34. Changes in the glycogen, pyruvate and lactate levels in the liver during exercise (circles represent means; vertical lines indicate standard error).


GLYCOGEN
PYRUVATE
LACTATE
LACTATE, mg/100gm wet wt.
PYRUVATE, mg/100gm wet wt.
GLYCOGEN, mg/100gm wet wt.
105
90
75
60
45
30
15
0
1.05
0.90
0.75
0.60
0.45
0.30
0.15
0
1,000
970
940
910
880
850
820
790
0
5
10
15
20
PERIOD OF EXERCISE (MINUTES)

after which a decline was noted. At the end of 20 minutes of muscular activity the blood pyruvate concentration was 0.308 mg/100 ml (Table 18 , Fig. 33).

From a value of 13.455 mg/100 ml in the unexercised condition the lactate concentration in the blood began to rise as the fish was exercised. The sharpness with which the blood lactate level increased after 10 minutes was a pattern similar to that in the muscles. After 20 minutes of exercise the lactate concentration in the blood reached a level as high as 52.290 mg/100 ml. (Table 18, Fig. 33).

IV. GLYCOGEN, PYRUVATE AND LACTATE CONCENTRATIONS OF THE LIVER.

In the course of present study, the glycogen concentration of the liver did not seem to follow any regular pattern during exercise. From a value of 974.666 mg/100 g in the resting condition the liver glycogen was found to decline when the fish was exercised for 5 minutes. The concentration seemed to rise when the period of exercise was extended to 10 minutes. Beyond this period, however, the glycogen content of the liver continued to fall, reaching a value of 852.266 mg/100 g by the end of 20 minutes of exercise (Table 19 , Fig. 34).

The concentration of pyruvate in the liver varied within a narrow range during the entire period of exercise. In the unexercised state, the liver pyruvate content was 0.500 mg/100 g.

This value continued to rise with the duration of muscular activity, attaining a peak level of 0.658 mg/100 g by the end of 10 minutes. Continuation of muscular activity after this period was characterized by a gradual decrease in the liver pyruvate. Towards the end of 20 minutes of exercise the values were found to have declined to 0.516 mg/100 g (Table 19 , Fig.34).

The lactate content of the liver, from a resting value of 15.615 mg/100 g appeared to rise with the increase in the period of exercise. A level of 52.290 mg/100 g was reached when the duration of exercise was extended to as much as 20 minutes (Table 19 , Fig.34).

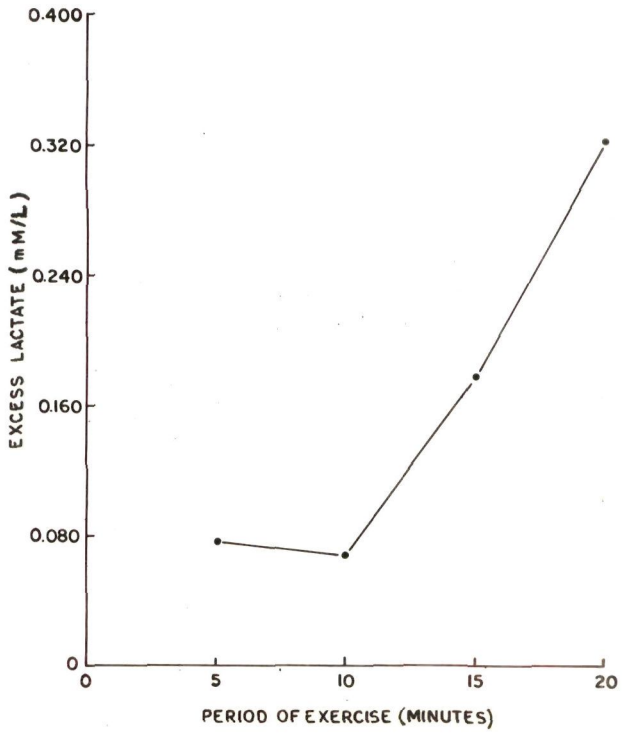
DISCUSSION

Inasmuch as the metabolism of protein and fat is essentially a slow process, glycolysis is the preponderant metabolic phenomenon in the body to meet the contingent energy requirements of increased muscular activity. As evident from its continuous decline during the period of exercise, the white muscle glycogen seemed to be the most easily utilisable source of energy. Considerable rise in the lactate levels in the white muscle during muscular activity further supported this view. The period of exercise which brought about a considerable depletion of white muscle glycogen produced little change in the glycogen content of the dark muscle, yet an enormous increase in its lactate content indicated that much carbohydrate has in fact been

metabolized there. It was, therefore, evident that the dark muscle was capable of resynthesizing glycogen very rapidly. The amount of glycogen depleted in this tissue during muscular activity, started replenishing at a very fast rate so that at the end of the exercise period the percentage of fall in the glycogen level of the dark muscle was relatively very small as compared to that of the white muscle. A corollary to this finding was apparent in the earlier observations of Mittenberger and Maciuc (1965) on Cyprinus carpio.

The glycogen content of the liver did not seem to follow any definite pattern of variation during the course of 20 minutes of exercise. Black et al. (1962) also found too irregular a pattern in the liver glycogen levels of Salmo gairdneri to draw any conclusions as to the influence of muscular stress. Love (1970), while making a general review of the literature on the subject, commented that the liver, which is the chief storehouse of glycogen, is but little depleted by the period to which the fish was exercised during the present experiment. In some cases, however, the liver glycogen has been reported to increase, undoubtedly, through fish-to-fish variation. Miller et al. (1959) observed that the reduction in the liver glycogen of Salmo gairdneri was brought about only when the fish was made to swim for 24 hours instead of 15 minutes or so. On the basis of the results obtained during the present study it may be concluded that glycogen is utilised rapidly at the site of energy requirement, the muscles, and could be replaced there only by a

Fig. 35. The level of 'excess lactate' in the blood during exercise.


0.400
0.320
0.240
0.160
0.080
0
EXCESS LACTATE (mM/L)
0
5
10
15
20
PERIOD OF EXERCISE (MINUTES)

relatively slow mobilization in the liver.

The metabolism of muscle glycogen during exercise proceeded through the pyruvate stage to the production of large quantities of lactate. An initial increase in the pyruvate level during the early (first 10 minutes) period of exercise and its decline thereafter, just when the lactate level was on the rise (Figs. 31,32), indicated to the conversion of pyruvate into lactate.

As the glycogen degradation during exercise occurred in the muscles, the pyruvate and lactate were not produced in the blood itself but were released into it from the muscular tissues. Consequently, the blood levels of these metabolites reflected their concentrations in the muscle tissues. After attaining a peak value at 10 minutes the pyruvate level in the blood began to decline, while the lactate continued to accumulate. The persistence of considerable quantities of lactate in the blood seemed to be due to the low level of tissue oxygenation, as was expected during severe exercise, resulting in a high ratio of $NAD.H_2$ to NAD. This was one of the factors which was known to determine the lactate level. The lactate so formed in excess of the increase in pyruvate concentration was termed the 'excess lactate'. The excess lactate continued to rise as the period of exercise was increased beyond 10 minutes (Table 20, Fig. 35), and the tissue oxygenation remained insufficient to

provide for its aerobic oxidation to carbon dioxide and its elimination through the gills. Further, as the lactate concentration is reported to impair with the oxygen-combining power of haemoglobin in fish blood (Duddenbrock, 1938; Secondat, 1950; Black, 1953), it might have an additive effect on the anaerobic processes already going on in the body, leading further to a rise in the level of excess lactate.

Whatever little increase in the levels of pyruvate and lactate occurred in the liver of C. batrachus during the course of of 20 minutes of exercise seemed due to the transport of these substances through the blood. The present data, however, provided no clues as to their conversion into glycogen in the hepatic tissues.

SUMMARY

An attempt is made to establish the pattern of carbohydrate metabolism in a freshwater cat-fish, Clarias batrachus, in relation to muscular activity. Except for the considerable disparity observed in the glycogen levels of the pre- and post exercise conditions, the turnover of the pyruvate and lactate was found to follow a similar pattern in the dark and white muscles. The relatively little change in the dark muscle glycogen was attributed to its tempest resynthesis. Blood levels of pyruvate and lactate were a reflection of the changes in the concentrations of these metabolites in the muscles. A high ratio

of $NAD.H_2$ to NAD seemed to result in the accumulation of vast quantities of lactate in the blood. The level of excess lactate continued to rise with the period of exercise. Changes in the liver glycogen in relation to muscular activity were irregular.

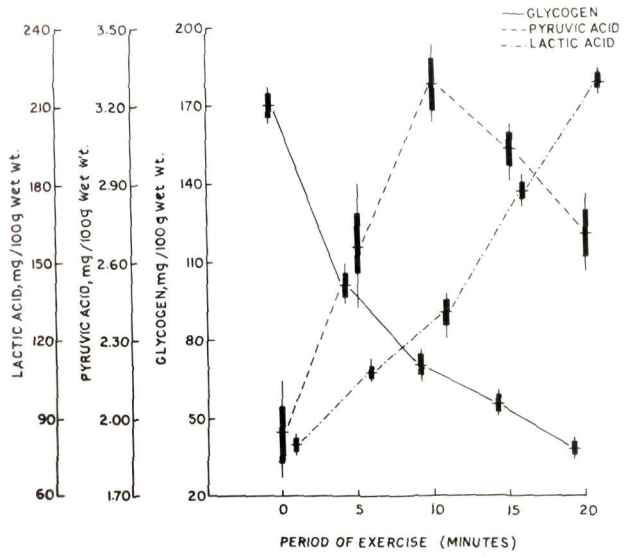
CHAPTER XII

SOME CHANGES IN THE CARBOHYDRATE METABOLISM IN *OPHIOCEPHALUS PUNCTATUS* BLOCH IN RELATION TO MUSCULAR ACTIVITY

INTRODUCTION

In addition to considerable literature on the effect of muscular activity on the physiological and biochemical processes in the skeletal muscle mass, in general, evidences distinguishing the changes occurring between the dark and white muscles were also cited in the previous chapter. The present study, besides describing the dynamics of the change in the various metabolites of the white muscle , deals with the metabolic changes in the liver, the main site for glycogen storage, in response to muscular activity in *Ophiocephalus punctatus* Bloch. This species was preferred because the proportion of dark muscle, relative to that of the white is extremely small and the total quantity of glycogen stored in it would be so little that it could hardly be assigned any major role in the physiological mechanism during muscular activity. The changes in the pyruvic and lactic acids of the blood were also studied with a view to elaborate the physiological significance of the exercise-induced alterations in the blood levels of 'excess lactate'.

Fig. 36. Changes in the glycogen; pyruvic and lactic acids in the white muscle of *O. punctatus* during exercise (Horizontal lines represent mean; vertical lines represent range and vertical bars indicate standard error).


GLYCOGEN
PYRUVIC ACID
LACTIC ACID
LACTIC ACID, mg/100g wet wt.
PYRUVIC ACID, mg/100g wet wt.
GLYCOGEN, mg/100g wet wt.
240
210
180
150
120
90
60
3.50
3.20
2.90
2.60
2.30
2.00
1.70
200
170
140
110
80
50
20
0
5
10
15
20
PERIOD OF EXERCISE (MINUTES)

MATERIALS AND METHODS

Methods for the procurement and maintenance of fishes, exercising them and sampling of their tissues and blood as well as the chemical analyses were the same as described under 'Procedure and Methodology' (pages 9 - 33). The changes in the levels of the chemical constituents in relation to muscular activity were tested for statistical significance by students' t-test.

RESULTS

GLYCOGEN AND PYRUVIC AND LACTIC ACIDS IN THE WHITE MUSCLE.

The glycogen content of the white muscle in the unexercised condition of *C. punctatus* was recorded to be 170.708 mg/100 g. This value continued to decline with the period of exercise. and by the end of 20 minutes a significant ($P < 0.01$) fall of about 77% was evident (Table 21 , Fig. 36).

The pyruvic acid, from a value of 1.941 mg/100 g in the resting condition of the fish, maintained a steady rise during the first 10 minutes of muscular activity, till it attained a value of 3.275 mg/100 g. This increase was significant at 1% level of probability. After 10 minutes onward the level of pyruvic acid continued to decline, reaching a value of 2.700 mg/100 g by the end of 20 minutes of exercise (Table 21 , Fig. 36).

Fig. 37. Changes in the blood levels of pyruvic and lactic acids in O. mungotalus during exercise (Horizontal lines represent mean; vertical lines represent range and vertical bars indicate standard error).

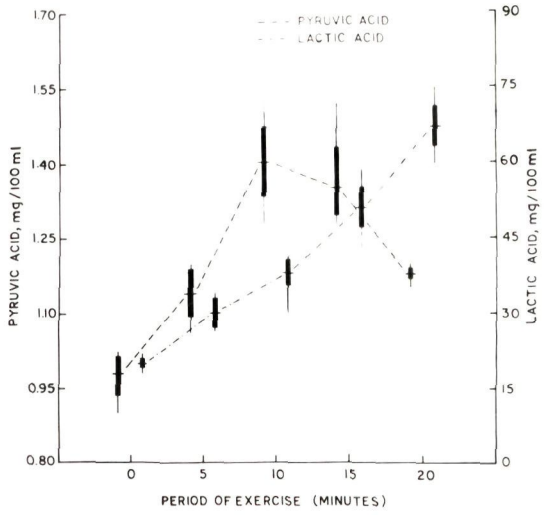
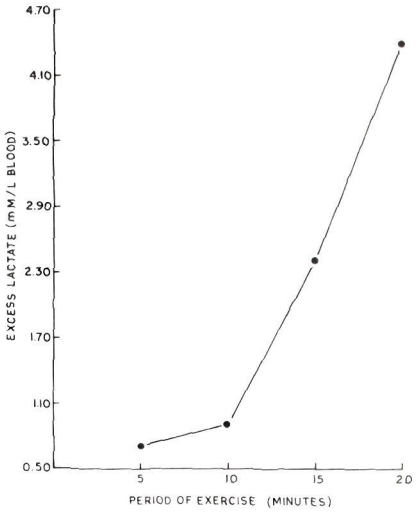

PYRUVIC ACID
LACTIC ACID
1.70
1.55
1.40
1.25
1.10
0.95
0.80
PYRUVIC ACID, mg/100 ml
90
75
60
45
30
15
0
LACTIC ACID, mg/100 ml
0
5
10
15
20
PERIOD OF EXERCISE (MINUTES)

Fig. 38. Changes in the blood levels of 'excess lactate' in *Q. punctatus* during exercise.


4.70
4.10
3.50
2.90
2.30
1.70
1.10
0.50
EXCESS LACTATE (m.M/L BLOOD)
5
10
15
20
PERIOD OF EXERCISE (MINUTES)

From a resting level of 30.367 mg/100 g, the concentration of lactic acid in the white muscle increased gradually during the first 10 minutes of exercise, while in the succeeding period the value began to rise rather sharply. A significant ($P < 0.01$) rise to 217.710 mg/100 g was observed when the period of exercise was exercise was extended to 20 minutes (Table 21 , Fig. 36).

PYRUVIC AND LACTIC ACIDS IN THE BLOOD.

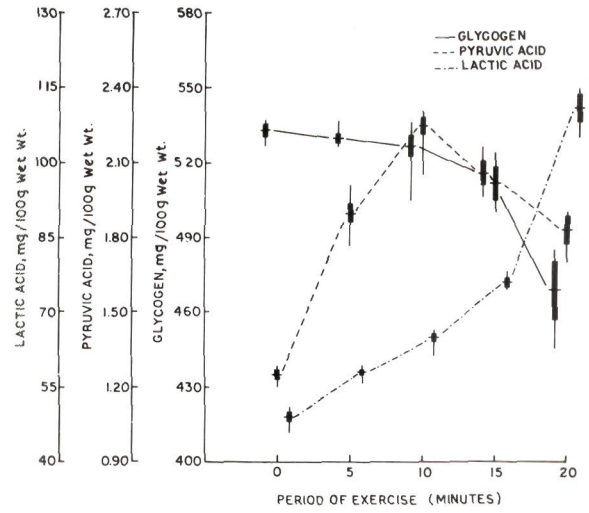
From a pre-exercise level of 0.903 mg/100 ml the concentration of pyruvic acid increased significantly ($P < 0.10$) to a value of 1.400 mg/100 ml and, thereafter, the level started declining, though did not reach the unexercised state (Table 22 , Fig. 37).

The lactic acid concentration of the blood which was 20.576 mg/100 ml in the resting state started increasing gradually during the first 10 minutes of muscular activity but took a sharp turn when the period of exercise was further extended. The concentration seemed to have risen significantly ($P < 0.01$) by the close of 20 minutes of muscular activity (Table 22 , Fig. 37).

EXCESS LACTATE

The gradual increase in the blood level of excess lactate during the first 10 minutes of exercise gave the way to a very abrupt increase in the subsequent period and this continued with the duration of exercise (Table 23 , Fig. 38). After 20 minutes, the excess lactate increased to about six-fold of its level in the early stage of exercise.

Fig. 39. Changes in the glycogen; pyruvic and lactic acids in the liver of O. punctatus during exercise (Horizontal lines represent mean; vertical lines represent range and vertical bars indicate standard error).


GLYCOGEN
PYRUVIC ACID
LACTIC ACID
LACTIC ACID, mg/100g Wet Wt.
PYRUVIC ACID, mg/100g Wet Wt.
GLYCOGEN, mg/100g Wet Wt.
130
115
100
85
70
55
40
2.70
2.40
2.10
1.80
1.50
1.20
0.90
580
550
520
490
460
430
400
0
5
10
15
20
PERIOD OF EXERCISE (MINUTES)

GLYCOGEN AND PYRUVIC AND LACTIC ACIDS IN THE LIVER.

From a resting value of 535.000 mg/100 g, very little change seemed to occur in the liver glycogen content of *C. punctatus* in the first 15 minutes of muscular activity, but, thereafter, the value declined ($P < 0.05$) to 469.200 mg/100 g, a fall of about 12% from its pre-exercise level (Table 24, Fig. 39).

The resting value of liver pyruvic acid was 1.250 mg/100 g. This value gradually increased ($P < 0.01$) to 2.250 mg/100 g after the fish was exercised for 10 minutes. Prolongation of the period of muscular activity was, however, characterised by a decline in the pyruvic acid content, though it did not attain the pre-exercise level (Table 24, Fig. 39).

The pre-exercise level of liver lactic acid (49.067 mg/100 g) increased slowly during the first 10 minutes of exercise but markedly in the subsequent period. A level of 111.080 mg/100 g was found to have reached when the duration of exercise was extended to 20 minutes (Table 24, Fig. 39). This was significantly higher ($P < 0.01$) than the resting level.

DISCUSSION

Muscular activity is known to be one of the intrinsic modifiers of carbohydrate metabolism in the body. The kinetics of the metabolic changes can be readily measured during the period of performance by fishes, of the strenuous exercise, where

emphasis is laid on the transient state rather than the steady state levels of metabolites.

Considerable depletion of white muscle glycogen, together with an immense increase in the lactic acid concentration, was evidently the result of the utilisation of the bulk of the carbohydrate in this tissue. The lactic acid/glycogen ratio, which is regarded as a measure of carbohydrate mobilisation during exercise (Black, 1962) was as high as 5.588 as compared to that in other teleostean species (*C. mrigal*), having conspicuous proportion of dark muscle, where it was 3.255, after the same period of exercise (20 minutes). Further, the glycogen content of the liver, which did not seem to follow any regular pattern of change during the course of 20 minutes of muscular activity in *C. mrigal*, was found to decline in *O. punctatus*. Obviously, due to the reduction of dark muscle the metabolic requirements of increased muscular activity were met at the expense of the glycogen contents of the white muscle and the liver, particularly of the former.

As in case of *C. mrigal*, the process of glycolysis in *O. punctatus* during muscular stress followed the 'Embden-Meyerhof-Parnas' (EMP) pathway, which resulted in the formation of large quantities of lactic acid through the intermediate stage of pyruvic acid.

The pattern of turnover of the various metabolites related to the various time-periods of exercise was also strikingly similar

in the two species of fish investigated: the first 10 minutes of muscular activity were characterised by an abrupt rise in the level of pyruvic acid while the subsequent period was associated with a gradual decline in the concentration of this metabolite, and a reciprocal increase in the level of lactic acid, indicating that conversion of pyruvic acid into lactic acid was started by then.

Inasmuch as the formation of pyruvic and lactic acids in the muscles was followed by their release into the blood stream, the alterations in the blood levels of these constituents reflected corresponding changes in their concentrations in the muscles. Hence, the pyruvic acid content of the blood, after attaining the peak value by the end of 10 minutes, began to subside, while the lactic acid started accumulating. The production of lactic acid has been known to depend on the concentration of pyruvic acid and the ratio of $NAD.H_2$ to NAD, and since the transformation of $NAD.H_2$ into NAD has been regarded to be a very slow process, the ratio $NAD.H_2/NAD$ could go on increasing with the period of muscular activity. Under the conditions of oxygen deficiency, as they prevail during the course of muscular activity, the quantity of lactic acid formed in excess of the increase in the level of pyruvic acid, and evidently due to the high value of $NAD.H_2/NAD$, was termed the excess lactate (Huckabee, 1958). The high levels of lactic acid which exert their inhibitory effect on the circulatory efficiency of the fish blood (Duddenbrock, 1938; Secondat,

1950; Mack, 1958) lead to further accumulation of excess lactate.

The rise in the levels of pyruvic and lactic acids in the liver of G. punctatus with the duration of muscular activity could partly be due to the transport of these substances through the blood and partly to the actual mobilization of glycogen there. The present study, however, did not lend any support as to the conversion of these metabolites into glycogen in the liver.

SUMMARY

The pattern of immediate changes in the carbohydrate metabolism was studied during muscular activity in Ophicephalus punctatus Bloch. The glycolytic pathway was found to follow the EMP - scheme. In this species, where the proportion of dark muscle was highly reduced, the metabolic requirements of increased muscular activity seemed to be met chiefly at the expense of white muscle glycogen, though a little depletion of liver glycogen did occur. The variations in the blood levels of pyruvic and lactic acids seemed a direct consequence of changes in these metabolites in the muscle. Prolongation of the period of muscular activity was characterized by a steady increase in the level of 'excess lactate'.

CHAPTER XIII

NUCLEIC ACID TURNOVER IN THE DARK AND WHITE MUSCLES OF TWO SPECIES OF CARPS, *LABEO CALBASU* (HAM.) AND *PUNTIUS SARANA* (HAM.) DURING GROWTH IN PRE-MATURITY STAGES

INTRODUCTION

Though it is evident from the central dogma of molecular biology that nucleic acids play significant roles in the organization of definite patterns of growth and metabolism through the synthesis of specific proteins, little information exists on the dynamics of their turnover in fish tissues during the process of growth. This chapter provides a comparative account of ribose nucleic acid (RNA) and deoxyribose nucleic acid (DNA) concentrations in the dark and white muscles and their changes during growth in the pre-maturity phase of two teleostean species, namely, *Labeo calbasu* (Ham.) and *Puntius sarana* (Ham.). The new informations emerged from the present studies have been interpreted in the light of the anatomical and physiological characteristics of the two types of muscles.

MATERIALS AND METHODS

Methods for the procurement of fish specimens of given lengths, muscle sampling, RNA and DNA determination and for the statistical elucidation of the data were the same as given under 'Procedure and Methodology' (pages 9-33).

RESULTS

The values of RNA and DNA in the dark and white muscles of *Labeo calbasu* and *Puntius sarana* are given in Table 25. It is evident from the table that in the specimens of both the size-groups examined, the concentrations of RNA and DNA in the dark muscle were significantly higher ($P < 0.001$) than in the white muscle, and that growth in the pre-maturity stage was accompanied by an increase in RNA and a decrease in DNA concentration of the two types of muscles. From the data it can be calculated that the RNA ratios in the II size-group to I size-group were greater than 0.5, in both the muscles of the two teleostean species. Dark and white muscles, however, showed differences in this ratio (Table 26).

DISCUSSION

Since concentration of RNA in a tissue indicates the level of metabolic activity (Leslie, 1955), the higher ($P < 0.001$) concentration of RNA in dark muscle implies greater metabolic activity in this tissue. Some of the earlier biochemical and physiological observations (Brockhan, 1956, 1959; Mori *et al.*, 1956; Tsuchiya and Kunii, 1960; Bareta, 1961) have also revealed that in contrast to white muscle, which is largely concerned with mechanical functions, dark muscle performs a great variety of metabolic functions, resembling the liver in many respects.

The greater ($P < 0.001$) concentration of RNA in dark muscle in comparison to that in white, is perhaps due to a larger number of cells contained per unit weight of this tissue. It has been reported that the cells of dark muscle are smaller than those of white (Thurston and Mackaster, 1960; George, 1962; Buttkus, 1963, Nishihara, 1967) and, therefore, a unit weight of dark muscle contains a greater number of cells, than an equal weight of white muscle. That the concentration of RNA is related to the number of cells present in a given weight of tissue sample is extensively reviewed by Leslie (1955).

Since increase in food intake elevates the RNA concentration of tissues (Brachet, 1955; Leslie, 1955; Bulow, 1970), the rise in the values of RNA in both the dark as well as the white muscle during growth may perhaps be related to the dietary factor. That there occurs increase in food consumption during growth in early life to meet the rising metabolic requirements of fish is indicated by the work of Love (1970). The increase in the concentration of RNA, an organiser of protein synthesis (Bulow, 1970), seems to be necessity for an active synthesis of protein in the growing tissues of the body during the pre-maturity stage, which is regarded as the period of most active growth of the fish.

Insofar as growth in body-length of fish proceeds through increase in the sizes of muscle cells rather than their number, it can be expected that RNA concentration should reduce to half once the fish doubles its body length, because this results in

halving the number of cells contained in the given weight of the muscle (Love, 1970). However, in the present study the DNA concentrations were not observed to be halved. This is evidenced from the fact that when the ratio of DNA in the muscle of larger size-group (II) to that in smaller size-group (I) was calculated, it was found to be greater than 0.5 in each case. Evidently, some synthesis of DNA occurs in muscle cells during growth. From the investigations of Love (1958) it appears as if this DNA synthesis in cells is necessary to 'control' the increasing cytoplasmic volume in growing cells.

The differences in DNA ratios of the dark and white muscles of the fish clearly reveal that the cells in the two muscles differ not only in their growth rate but also in synthesizing the amounts of DNA.

SUMMARY

Attempt was made to investigate the concentrations of RNA and DNA in the dark and white muscles, together with the turnover of these constituents during growth in the pre-maturity phase, in *Labeo calbasu* and *Puntius sarana*. The dark muscle of both the species contained significantly higher ($P < 0.001$) concentrations of RNA and DNA than the white. An increase in the concentration of RNA and a decrease in that of DNA seemed to occur in both the muscles during growth in the pre-maturity phase. The

apparent decline in DNA concentration per unit weight of dark and white muscle during growth, appeared to be compensated, to some extent, by the synthesis of more DNA in cells, presumably to 'control' the growing cytoplasmic volume. Cells of the dark and white muscles differred not only in their growth rates but also in DNA-synthesis.

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SIZES (AXIS IN *Sphicephalus punctatus*

LENGTH OF FISH (mm)	MEAN OF LATERAL SEPTUM FROM SURFACE TO SAGITTAL AXIS (mm)				
	RANGE	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION
120	- 7.60	7.52	0.037	0.0068	1.1037
170	-11.20	10.96	0.088	0.0388	1.7974
220	-14.80	14.68	0.037	0.0068	0.5653
248	-18.60	17.50	0.396	0.7849	5.0628

TABLE 2

MEASUREMENTS OF 4th AND 21st MYOTOMES OF *Ophiocephalus punctatus*

LENGTH OF FISH (mm)	AGE OF FISH (YEARS)	THICKNESS OF 4th MYOTOME (mm)					THICKNESS OF 21st MYOTOME (mm)				
		RANGE	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION	RANGE	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION
120	0+	2.00–2.10	2.04	0.020	0.0028	2.5980	1.30–1.70	1.50	0.052	0.0191	9.2266
170	1+	2.90–3.10	3.04	0.020	0.0028	1.7434	2.18–2.28	2.24	0.015	0.0017	1.8571
220	2+	3.80–4.00	3.92	0.036	0.0090	2.4234	2.83–2.93	2.89	0.015	0.0017	1.4394
248	3+	4.56–4.61	4.58	0.006	0.0002	0.3711	3.30–3.48	3.39	0.020	0.0028	1.5634

TABLE 3

MYOSOMATIC INDICES AND THEIR INTRASPECIFIC VARIATION IN *Ophiocephalus punctatus*

LENGTH OF FISH (mm)	AGE OF FISH (YEARS)	MYOSOMATIC INDEX									
		PERTAINING TO 4th MYOTOME					PERTAINING TO 21st MYOTOME				
		RANGE	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION	RANGE	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION
120	0+	1.700					1.250				
170	1+	1.788					1.317				
			1.778	0.030	0.0036	3.374		1.311	0.023	0.0021	3.546
220	2+	1.781					1.313				
248	3+	1.846					1.366				

TABLE 4

GROWTH OF THE MYOTOMES OF *Ophiocephalus punctatus*

LENGTH OF FISH (mm)	AGE OF FISH (YEARS)	MEAN THICKNESS OF MYOTOMES (mm)		GROWTH RATES OF MYOTOMES (PER YEAR)		RATIO OF THE RATES OF GROWTH				MEAN RELATIVE GROWTH OF 4th MYOTOME IN SUCCESSIVE YEARS
		4th MYOTOME	21st MYOTOME	4th MYOTOME	21st MYOTOME	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION	
120	0+	2.04	1.50	2.04	1.50					—
170	1+	3.04	2.24	1.00	0.74					49.01
						1.346	0.012	0.00031	1.3187	
220	2+	3.92	2.89	0.88	0.65					28.94
248	3+	4.58	3.39	0.66	0.50					16.83

TABLE 5

CONCENTRATION OF BIOCHEMICAL CONSTITUENTS IN THE THIGH AND TAIL MUSCLE OF THE FILLET OF *Cubiceps pauciradiatus*

ANATOMICAL LOCATION	PROTEIN (%)	FAT (%)	WATER (%)	ASH (%)	TOTAL CALCIUM (%)	GLYCOGEN (mg/100 g)	CHOLESTEROL (mg/100 g)	EPA (µg/100 mg)	DHA (µg/100 mg)	ENERGY VALUE (Calories/100 g)
THIGH	18.499 ± 0.406	1.040 ± 0.101	76.933 ± 0.314	1.200 ± 0.152	2.537 ± 0.898	160.366 ± 6.505	114.000 ± 19.425	100.672 ± 2.476	12.213 ± 0.464	95.060 ± 2.646
TAIL	19.739 ± 0.137	1.706 ± 0.146	73.433 ± 0.523	1.533 ± 0.176	3.507 ± 0.535	233.325 ± 9.362	173.000 ± 12.005	156.823 ± 3.855	23.469 ± 1.113	111.510 ± 2.231

The values are mean ± standard error.

TABLE 6

CONCENTRATIONS OF SOME CHEMICAL CONSTITUENTS IN THE DARK AND WHITE MUSCLES AND LIVER OF

Clarias mossr

TISSUE	PROTEIN (%)	FAT (%)	WATER (%)	ASH (%)	TOTAL CARBOHYDRATES (%)	GLYCOGEN (mg/100 g)	CHOLESTEROL (mg/100 g)	EPA (µg/100 mg)	DHA (µg/100 mg)
WHITE MUSCLE	16.783 ± 0.344	1.000 ± 0.086	79.033 ± 0.087	1.416 ± 0.043	2.767 ± 0.395	235.308 ± 7.607	152.000 ± 14.468	88.278 ± 1.826	11.749 ± 0.669
DARK MUSCLE	15.278 ± 0.158	2.850 ± 0.086	76.400 ± 0.360	1.283 ± 0.016	4.188 ± 0.216	330.650 ± 5.308	271.333 ± 10.477	120.787 ± 2.416	27.060 ± 1.146
LIVER	11.656 ± 0.172	8.766 ± 0.185	70.433 ± 0.577	1.050 ± 0.028	8.093 ± 0.321	942.933 ± 16.345	670.000 ± 24.000	529.672 ± 18.411	75.885 ± 1.532

The values are Mean ± Standard Error.

TABLE 7

PROXIMATE CHEMICAL CONSTITUENTS OF THE LATERAL MUSCLE OF *Channocephalus striatus* BELONGING TO DIFFERENT AGE GROUPS

AGE GROUP	LENGTH RANGE (mm)	PROTEIN (%)				FAT (%)				WATER (%)				ASH (%)			
		MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION
0+	200-220	13.814	0.0174	0.00159	0.2888	0.569	0.0026	0.00004	0.0499	76.599	0.0192	0.00188	0.0566	1.340	0.0013	0.00001	0.2686
1+	300-320	13.171	0.0228	0.00265	0.4114	0.505	0.0017	0.00001	0.0311	76.802	0.0076	0.00029	0.0222	1.082	0.0102	0.00052	2.1256
2+	390-410	12.516	0.0219	0.00245	0.3954	0.356	0.0049	0.00014	0.0950	78.014	0.0570	0.01646	0.1644	1.205	0.0062	0.00020	1.1867
3+	440-460	11.282	0.0035	0.00007	0.0744	0.281	0.0067	0.00023	0.1350	79.924	0.0098	0.00051	0.0284	1.306	0.0053	0.00015	0.9494
4+	470-490	15.155	0.0035	0.00006	0.0527	0.679	0.0040	0.00009	0.0487	74.341	0.0035	0.00007	0.0115	1.558	0.0059	0.00003	0.3786
5+	500-520	14.989	0.0035	0.00007	0.0567	0.580	0.0031	0.00005	0.6913	74.983	0.0031	0.00005	0.0095	1.403	0.0040	0.00009	0.6913

TABLE 8

RNA AND PROTEIN CONCENTRATIONS IN THE FLESH OF *Ophiocephalus punctatus* DURING GROWTH

AGE (YEARS)	LENGTH RANGE (cm)	RNA (µg/100 mg)	PROTEIN (mg/100 mg)
0+	1 - 10	119.643±2.405	17.528±0.221
1+	11 - 14	100.195±1.718	16.226±0.312
2+	15 - 18	109.728±0.953	17.444±0.168
3+	19 - 20	137.852±3.302	18.050±0.186
4+	21 - 22	151.008±1.838	18.610±0.092
5+	23 - 24	158.158±1.321	18.797±0.186

The values are Mean ± Standard Error.

TABLE 9

RNA AND PROTEIN CONCENTRATIONS IN THE FLESH OF *Ophiocephalus punctatus* IN RELATION TO FILLET CONDITION FACTOR

BODY LENGTH (cm)	WEIGHT (g)	FILLET WEIGHT (g)	FILLET CONDITION FACTOR	RNA (µg/100 mg)	PROTEIN (mg/100 mg)
23.0	162	92.0	7.561	172.172	19.572
20.0	100	56.0	7.000	165.022	19.572
23.0	155	85.0	6.986	165.022	18.984
20.5	108	60.0	6.964	167.310	18.984
22.0	118	72.0	6.761	160.446	18.704
23.0	130	76.0	6.246	162.734	18.144
21.5	102	61.4	6.176	158.158	18.144
22.5	127	70.0	6.145	153.868	17.864
20.5	90	52.0	6.035	151.580	17.864
20.0	85	48.0	6.003	149.578	17.612
21.0	98	55.0	5.938	147.576	17.612
20.0	88	46.0	5.750	143.572	17.360
22.0	108	60.0	5.634	137.852	17.108
22.5	114	62.0	5.443	132.132	16.856
22.5	110	58.0	5.091	126.984	16.632

TABLE 10

CHANGES IN BODY WEIGHT AND FILLET CONDITION FACTOR OF *Heteropneustes fossilis* DURING STARVATION

PERIOD OF STARVATION (DAYS)	LENGTH OF FISH (cm)	WEIGHT OF FISH BEFORE STARVATION (g)	WEIGHT OF FISH AFTER STARVATION (g)	LOSS IN BODY WEIGHT (g)	% LOSS IN BODY WEIGHT		FILLET WEIGHT (g)	FILLET CONDITION FACTOR	
					RANGE	MEAN		RANGE	MEAN
	21.5	69.0	-	-	-	-	38.320	3.855	
0	24.0	83.0	-	-	-	-	50.234	3.633	3.682
	24.7	92.0	-	-	-	-	53.634	3.559	
	19.0	49.0	44.0	5.0	10.204		23.358	3.405	
10	23.0	68.0	61.0	7.0	10.294	11.710	25.990	2.136	2.771
	18.0	41.0	35.0	6.0	14.634		16.174	2.773	
	20.2	43.0	37.0	6.0	13.953		19.180	2.326	
20	21.0	60.2	53.0	7.2	11.960	13.199	26.746	2.888	2.471
	24.0	81.1	70.0	11.1	13.686		30.412	2.199	
	17.0	30.0	26.0	4.0	13.333		12.898	2.625	
30	21.5	35.0	27.5	7.5	21.428	16.246	19.500	1.962	2.189
	22.0	46.5	40.0	6.5	13.978		22.800	2.141	
	20.5	47.5	41.5	6.0	12.631		19.926	2.312	
40	18.0	30.5	24.0	6.5	21.311	19.208	10.060	1.724	1.957
	18.5	38.0	29.0	9.0	23.684		11.630	1.836	
	23.4	70.0	55.0	15.0	21.428		24.332	1.999	
50	24.0	73.0	57.0	16.0	21.917	20.975	24.150	1.746	1.749
	23.5	71.5	57.5	14.0	19.500		19.520	1.502	

TABLE 8

RNA AND PROTEIN CONCENTRATIONS IN THE MUSCLE OF *Ophiocephalus punctatus* DURING GROWTH

AGE (YEARS)	LENGTH RANGE (cm)	RNA (µg/100 mg)	PROTEIN (mg/100 mg)
0+	1 - 10	119.643±2.405	17.528±0.221
1+	11 - 14	100.195±1.718	16.226±0.312
2+	15 - 18	109.728±0.953	17.444±0.168
3+	19 - 20	137.852±3.302	18.050±0.186
4+	21 - 22	151.008±1.838	18.610±0.092
5+	23 - 24	158.158±1.321	18.797±0.186

The values are Mean ± Standard Error.

TABLE 9

RNA AND PROTEIN CONCENTRATIONS IN THE FLESH OF *Ophiocephalus punctatus* IN RELATION TO FILLET CONDITION FACTOR

BODY LENGTH (cm)	WEIGHT (g)	FILLET WEIGHT (g)	FILLET CONDITION FACTOR	RNA (µg/100 mg)	PROTEIN (mg/100 mg)
23.0	162	92.0	7.561	172.172	19.572
20.0	100	56.0	7.000	165.022	19.572
23.0	155	85.0	6.986	165.022	18.984
20.5	108	60.0	6.964	167.310	18.984
22.0	118	72.0	6.761	160.446	18.704
23.0	130	76.0	6.246	162.734	18.144
21.5	102	61.4	6.176	158.158	18.144
22.5	127	70.0	6.145	153.868	17.864
20.5	90	52.0	6.035	151.580	17.864
20.0	85	48.0	6.003	149.578	17.612
21.0	98	55.0	5.938	147.576	17.612
20.0	88	46.0	5.750	143.572	17.360
22.0	108	60.0	5.634	137.852	17.108
22.5	114	62.0	5.443	132.132	16.856
22.5	110	58.0	5.091	126.984	16.632

TABLE 11

ABSOLUTE CONCENTRATIONS OF PRINCIPAL CHEMICAL CONSTITUENTS AND ENERGY VALUES IN *Heteromeustes fossilis* DURING STARVATION

PERIOD OF STARVATION (DAYS)	PRINCIPAL CHEMICAL CONSTITUENTS (g)				ENERGY CONTENT (CALORIES)
	PROTEIN	FAT	WATER	ASH	
0	8.124	1.007	39.400	0.625	42.673
10	7.172	0.089	34.786	0.551	37.672
20	6.419	0.578	34.409	0.506	31.692
30	5.365	0.424	33.319	0.453	25.260
40	5.027	0.342	32.312	0.390	24.176
50	4.650	0.205	32.163	0.348	20.971

CHANGES IN THE PRINCIPAL CHEMICAL CONSTITUENTS AND ENERGY VALUE IN THE FLESH OF Heteropneustes fossilis DURING STARVATION

PERIOD OF STARVATION (DAYS)	PROTEIN (%)				FAT (%)				WATER (%)				ASH (%)				ENERGY VALUE (CALORIES)
	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION	MEAN	STANDARD ERROR	VARIANCE	COEFFICIENT OF VARIATION	
0	16.958	0.115	0.0400	11.793	2.767	0.029	0.0025	1.815	77.033	0.536	0.0299	1.171	1.350	0.076	0.0174	9.792	95.446
10	16.249	0.476	0.6822	5.084	2.014	0.121	0.0441	10.466	78.000	0.076	0.0174	0.167	1.250	0.028	0.0025	4.000	85.350
20	14.791	0.275	0.2275	3.225	1.334	0.065	0.0127	8.478	79.466	0.044	0.0057	0.096	1.166	0.044	0.0057	6.543	73.049
30	12.812	0.737	1.6352	9.982	1.014	0.042	0.0053	7.228	79.566	0.072	0.0156	0.158	1.083	0.044	0.0057	7.045	61.958
40	12.447	0.376	0.4220	5.222	0.848	0.124	0.0462	25.389	79.991	0.247	0.1040	0.536	0.966	0.016	0.0007	2.981	61.054
50	11.770	0.375	0.4225	5.527	0.521	0.096	0.0278	32.111	81.500	1.395	5.8370	2.965	0.883	0.033	0.0032	6.534	53.102

TABLE 13

DISTRIBUTION OF ACID AND ALKALINE PHOSPHATASES (BODANSKY UNITS) AND 5'-NUCLEOTIDASE (µg pi/ml/hr) IN THE DARK AND WHITE MUSCLES OF *C. mrigal* AND *Heteropneustes fossilis*

SPECIES	TYPE OF MUSCLE	ACID PHOSPHATASE	ALKALINE PHOSPHATASE	5'-NUCLEOTIDASE
		MEAN ± S.E.	MEAN ± S.E.	MEAN ± S.E.
C. mrigal	WHITE	0.393 ± 0.028	0.353 ± 0.055	11.331 ± 2.553
	DARK	0.548 ± 0.059	0.707 ± 0.046	8.853 ± 2.153
H. fossilis	WHITE	0.279 ± 0.068	0.319 ± 0.040	11.686 ± 3.071
	DARK	0.454 ± 0.064	0.437 ± 0.050	9.207 ± 2.478

The values are Mean ± Standard Error.

TABLE 14

ALKALINE PHOSPHATASE ACTIVITY IN THE DARK, WHITE MUSCLES AND THE LIVER OF *Clarias batrachus* AND *Heteropneustes fossilis*

SPECIES	ALKALINE PHOSPHATASE ACTIVITY (BODANSKY UNITS)		
	WHITE MUSCLE	DARK MUSCLE	LIVER
C. batrachus	0.840±0.032	1.050±0.011	0.610±0.030
H. fossilis	0.795±0.013	0.978±0.025	0.648±0.028

The values are Mean ± Standard Error

TABLE 15

AMINO ACID INHIBITION OF ALKALINE PHOSPHATASE ACTIVITY IN THE DARK AND WHITE MUSCLES, AND THE LIVER OF *Clarias mossr* AND *Heterobranchus fossilis*

SPECIES	AMINO ACIDS (10 mM)	ALKALINE PHOSPHATASE ACTIVITY (BODANSKY UNITS)		
		WHITE MUSCLE	DARK MUSCLE	LIVER
C. mossr	L-phenylalanine	0.206±0.012	0.932±0.012	0.450±0.008
	L-methionine	0.696±0.015	0.570±0.017	0.532±0.017
	L-arginine	0.248±0.028	0.858±0.017	0.530±0.013
	L-valine	0.267±0.043	0.932±0.012	0.564±0.024
H. fossilis	L-phenylalanine	0.230±0.018	0.846±0.015	0.444±0.011
	L-methionine	0.716±0.024	0.614±0.016	0.475±0.017
	L-arginine	0.356±0.020	0.774±0.017	0.492±0.025
	L-valine	0.434±0.032	0.636±0.020	0.583±0.026

The values are Mean ± Standard Error.

TABLE 16

CHANGES IN THE GLYCOGEN, PYRUVATE AND LACTATE IN THE WHITE MUSCLE OF *Clarias batrachus* DURING MUSCULAR ACTIVITY

PERIOD OF EXERCISE (MINUTES)	GLYCOGEN (mg/100 g)	PYRUVATE (mg/100 g)	LACTATE (mg/100 g)
0	246.500±6.104	1.141±0.036	78.660±0.986
5	124.241±2.086	4.000±0.066	178.155±5.520
10	92.791±3.164	4.433±0.050	196.965±4.875
15	79.616±3.312	4.108±0.009	220.635±4.365
20	70.833±0.566	3.775±0.050	230.625±8.674

The values are Mean ± Standard Error.

TABLE 17

CHANGES IN THE GLYCOGEN, PYRUVATE AND LACTATE IN THE DARK MUSCLE OF *Clarias batrachus* DURING MUSCULAR ACTIVITY

PERIOD OF EXERCISE (MINUTES)	GLYCOGEN (mg/100 g)	PYRUVATE (mg/100 g)	LACTATE (mg/100 g)
0	333.483±2.707	0.908±0.006	61.605±0.974
5	306.283±2.836	2.975±0.043	121.005±6.080
10	302.033±2.833	3.100±0.026	133.380±5.536
15	294.950±4.907	2.825±0.026	159.075±3.786
20	285.033±2.836	2.650±0.050	175.860±3.392

The values are Mean ± Standard Error.

TABLE 13

CHANGES IN THE BLOOD LEVELS OF PYRUVATE AND LACTATE IN *Clarias mamar* DURING MUSCULAR ACTIVITY

PERIOD OF EXERCISE (MINUTES)	PYRUVATE (mg/100 ml)	LACTATE (mg/100 ml)
0	0.208±0.016	13.455±0.196
5	0.375±0.026	31.770±0.443
10	0.433±0.016	34.740±0.952
15	0.416±0.016	44.685±0.809
20	0.308±0.016	52.290±0.818

The values are Mean ± Standard Error.

CHANGES IN THE GLYCOGEN, PYRUVATE AND LACTATE IN THE LIVER OF *Clarias batrachus* DURING MUSCULAR ACTIVITY

PERIOD OF EXERCISE (MINUTES)	GLYCOGEN (mg/100 g)	PYRUVATE (mg/100 g)	LACTATE (mg/100 g)
0	974.666±11.994	0.500±0.028	15.615±0.671
5	961.066±16.962	0.600±0.026	33.120±0.472
10	988.266± 4.532	0.658±0.016	36.180±0.623
15	933.866±11.994	0.575±0.026	40.275±1.029
20	852.266±11.994	0.516±0.016	41.670±0.862

The values are Mean ± Standard Error.

TABLE 2C

CHANGES IN THE BLOOD LEVELS OF EXCESS LACTATE IN *Clarias batrachus* DURING MUSCULAR ACTIVITY

PERIOD OF EXERCISE (MINUTES)	EXCESS LACTATE (ml/L BLOOD)
5	0.076
10	0.069
15	0.179
20	0.324

CHANGES IN THE GLYCOGEN, PYRUVIC AND LACTIC ACIDS IN THE WHITE MUSCLE OF *Ophiocephalus punctatus* DURING MUSCULAR ACTIVITY

DURATION OF EXERCISE (MINUTES)	GLYCOGEN (mg/100 g)	PYRUVIC ACID (mg/100 g)	LACTIC ACID (mg/100 g)
0	170.708±4.122	1.94±0.109	80.867±1.621
5	101.850±4.696	2.675±0.137	108.782±1.993
10	70.975±3.564	3.275±0.086	130.079±4.362
15	55.675±2.208	3.025±0.065	176.556±2.741
20	38.958±1.351	2.700±0.086	217.710±1.901

The values are Mean ± Standard Error.

TABLE 22

CHANGES IN THE BLOOD LEVELS OF PYRUVIC AND LACTIC ACIDS IN *Onithocephalus punctatus* DURING MUSCULAR ACTIVITY

PERIOD OF EXERCISE (MINUTES)	PYRUVIC ACID (mg/100 ml)	LACTIC ACID (mg/100 ml)
0	0.983±0.041	20.576±0.718
5	1.141±0.057	30.649±2.492
10	1.400±0.072	38.131±3.740
15	1.358±0.084	51.514±4.361
20	1.183±0.016	67.054±4.610

The values are Mean ± Standard Error.

TABLE 23

CHANGES IN THE BLOOD LEVEL OF EXCESS LACTATE IN *Cyprinus carpio punctatus* DURING MUSCULAR ACTIVITY

PERIOD OF EXERCISE (MINUTES)	EXCESS LACTATE (ml/L BLOOD)
5	0.716
10	0.924
15	2.414
20	4.413

TABLE 24

CHANGES IN THE GLYCOGEN, PYRUVIC AND LACTIC ACIDS IN THE LIVER OF *Ophiocephalus punctatus* DURING MUSCULAR ACTIVITY

PERIOD OF EXERCISE (MINUTES)	GLYCOGEN (mg/100 g)	PYRUVIC ACID (mg/100 g)	LACTIC ACID (mg/100 g)
0	533.800±3.399	1.250±0.024	49.067±1.372
5	530.400±3.399	1.900±0.072	58.276±0.863
10	527.266±6.350	2.250±0.049	65.193±1.726
15	516.800±5.889	2.025±0.072	76.119±1.006
20	469.200±12.292	1.833±0.066	111.080±3.045

The values are Mean ± Standard Error.

RNA AND DNA CONCENTRATIONS IN DARK AND WHITE MUSCLES OF CARPS OF DIFFERENT BODY LENGTHS

(MEAN VALUES ARE EXPRESSED ON FRESHWEIGHT BASIS: SAMPLE SIZE IS GIVEN IN PARENTHESES)

SPECIES OF FISH	SIZE-GROUP	LENGTH OF FISH (mm)	DARK MUSCLE		WHITE MUSCLE	
			RNA (µg/100 mg)	DNA (µg/100 mg)	RNA (µg/100 mg)	DNA (µg/100 mg)
	I	70	124.859±2.562 (4)	26.066±0.565 (6)	77.105±1.283 (5)	11.262±0.438 (6)
Labeo calbasu	II	140	139.739±1.902 (5)	20.598±0.564 (5)	98.145±1.500 (5)	8.877±0.256 (6)
	I	60	104.485±2.266 (6)	16.291±0.320 (4)	65.589±2.394 (5)	7.578±0.587 (4)
Puntius sarana	II	120	119.976±1.590 (6)	12.997±0.551 (4)	76.648±2.411 (4)	4.497±0.226 (4)

± S.E. = Standard error.

TABLE 26

DNA-RATIOS IN THE DARK AND WHITE MUSCLES OF THE CARPS DURING DOUBLING OF BODY LENGTH (*Labeo calbasu*, 140:70 mm; *Puntius sarana*, 120:60 mm).

SPECIES	DARK MUSCLE	WHITE MUSCLE
L. calbasu	0.790	0.788
P. sarana	0.797	0.593